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EFFECTS OF SOIL TYPE AND ADULT SIZE ON MATING PROPENSITY AND REPRODUCTIVE OUTPUT IN TWO POPULATIONS OF THE LAND SNAIL *ARIANTA ARBUSTORUM* (LINNAEUS)

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ABSTRACT

Life-history traits in terrestrial gastropods may be influenced by both abiotic and biotic factors. This study examines the effects of soil type and adult size (shell volume) on mating propensity and female and male reproductive output (number and mass of eggs, number of sperm delivered and spermatophore mass) in individuals of the simultaneous hermaphrodite land snail *Arianta arbustorum* from two populations kept both on calcium-(Ca-)rich and Ca-poor soil. Snails from the two populations differed in adult size, relative shell growth, mating propensity and egg size. Furthermore, in both populations the number of egg batches deposited, egg size and spermatophore size scaled allometrically with shell volume, but not the total number of eggs produced and number of sperm delivered. Independent of population and shell size, the type of soil on which the snails were maintained influenced mating propensity, the total number of eggs produced and the mass of the albumen gland (another measure of female reproductive output). The mating propensity was higher and the total number of eggs produced was larger in snails kept on Ca-poor soil than in individuals reared on Ca-rich soil. This surprising finding could be explained by the fact that the Ca-poor soil used in the experiment still contained enough Ca to allow reproduction, and that the snails ingested Ca through the food consumed (lettuce grown on Ca-rich soil was available *ad libitum*). Moreover, the Ca-rich soil could contain minerals or (unknown) substances which discourage reproduction in *A. arbustorum*. Our study highlights the complexities faced when interpreting differences in the life history of gastropods. Explaining interpopulational differences in life-history patterns requires not only the understanding of the influence of snail origin, but also an understanding of the effects of shell size, substratum type (soil type), food and local climate.

Key words: *Arianta arbustorum*, calcium availability, egg size, reproductive allocation, simultaneous hermaphrodite, sperm number.

INTRODUCTION

Life-history theory predicts that a species occurring in different environments exhibits interpopulational variation in life-history traits as a result of different selection pressures (Stearns, 1992). However, observed local differences in life histories may also result from founder effects, genetic drift, and phenotypic plasticity (Calow, 1978; Caswell, 1983). Moreover, evolutionary interpretations of life-history patterns require a distinction to be made between genotypic and environmentally induced phenotypic variation, because life-history variation may also be the result of developmental plasticity or physiological acclimatization. It follows that observed variation in life history could simply mirror differences in habitat quality.

Reproductive resource allocation is a fundamental aspect of life history with profound ecological and evolutionary consequences (Stearns, 1992). Shelled gastropods strongly depend on calcium (Ca) as a major macronutrient constituent of their body (Dallinger et al., 2001). Besides reinforcing the shell, Ca is critical to a variety of functions in soft-tissue metabolism and reproduction (Tompa & Wilbur, 1977; Porcel et al., 1996). Most of the Ca absorbed by terrestrial gastropods may enter the animal's body via the epithelium of the intestine (Dexheimer, 1963; Beeby & Richmond, 2007). Because of seasonal needs for Ca, snails store

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this mineral mainly as calcium carbonate and possess high reallocation capacities (Fournié & Chétail, 1984). Ca can be mobilized readily from the intracellular storage site during periods of increased demand (Tompa & Wilbur, 1977). Ca provision to the eggs represents a major cost to the parent, and a variety of strategies are used to ensure sufficient Ca for the hatchling to build a shell (Tompa, 1980; Baur, 1994a). It is well established that soil type and calcium availability influence shell growth and female reproductive output (number of eggs laid) in terrestrial gastropods (Baur, 1994a; Heller, 2001). For example, egg production of *Helix pomatia* Linnaeus, 1758, exposed to acid soil was doubled when calcium carbonate was supplied during an experiment (Crowell, 1973). However, the potential effect of soil type on mating behaviour and the male reproductive output (spermatophore size and number of sperm delivered during copulation) have not been investigated in terrestrial gastropods.

Here we present the results of an experiment designed to examine whether mating propensity and sex-specific reproductive allocation in the simultaneous hermaphrodite land snail *Arianta arbustorum* (Linnaeus, 1758) are affected by the type of soil. In a reciprocal transplant experiment, snails from habitats with Ca-rich and Ca-poor soils were kept either on their original soil or on the other soil under laboratory conditions. In particular, we asked whether the origin of the snails, adult shell size and/or soil type influence the mating propensity, number of sperm delivered during copulation, and number of eggs laid within a season in *A. arbustorum*. We also examined whether the ratio of the resources allocated either to the male or female reproductive output is affected by the snails' origin, shell size, and soil type.

MATERIALS AND METHODS

Study Animals

Arianta arbustorum is common in moist habitats of northwestern and central Europe (Kerney & Cameron, 1979). The snail has determinate growth (shell breadth of adults 17–22 mm). Individuals become sexually mature at 2–4 years, and adults live another 3–4 years (maximum 14 years; Baur & Raboud, 1988). In the field, snails deposit one to three egg batches consisting of 20–50 eggs, per reproductive season (Baur & Raboud, 1988; Baur, 1990). In contrast to male fecundity (i.e., sperm expenditure), female fecundity (i.e., clutch size and the number of batches produced per season) is positively correlated with adult shell size (Baur, 1994a; Baur et al., 1998).

Mating in *A. arbustorum* includes elaborate courtship behaviour with optional dart shooting (i.e., the pushing of a calcareous dart into the mating partner's body), and lasts 2–8 h (Hofmann, 1923; Baur, 1992a). Copulation is reciprocal; after intromission, each snail transfers simultaneously one spermatophore (Haase & Baur, 1995). The spermatophore is formed and filled with sperm during copulation (Hofmann, 1923). It has a distinctive form consisting of a head, a body (sperm container with 800,000–4,000,000 spermatozoa), and a tail 2–3 cm long (Baur et al., 1998). Fertile sperm can be stored for more than one year (Baur, 1988). Mating is random with respect to shell size and different degrees of relatedness (Baur, 1992a; Baur & Baur, 1997). Individuals need at least eight days to replenish their sperm reserves after a successful copulation (Locher & Baur, 1999; Hänggi et al., 2002).

Paternity analysis in broods of wild-caught *A. arbustorum* showed a high frequency of multiple insemination (Baur, 1994b). A controlled laboratory experiment showed that one successful copulation per reproductive season is sufficient to fertilize all the eggs produced by an individual (Chen & Baur, 1993). However, there is a probability of 5–8% that a copulation will not lead to fertilization of eggs (no sperm transfer or transfer of infertile sperm; Chen & Baur, 1993).

Arianta arbustorum feeds on vascular plants and dead or senescent herbs, but the snails often supplement their vegetable diet with microorganisms, soil and carrion (Frömming, 1954; Grime & Blythe, 1969).

General Methods

Subadult *A. arbustorum* were collected from a locality with Ca-rich soil (Gurnigel) and a locality with Ca-poor soil (Zastler) in late April and early May 2002. Snails in the site with Ca-rich soil inhabited the embankment of a track in the subalpine forest near Gurnigel, 30 km south of Bern, Switzerland (46°45'N, 7°27'E; elevation 1,320 m above sea level), those in the site with Ca-poor soil the embankment of a forest road in the Black forest, Germany (47°54'N, 8°01'E; elevation 1,060 m). The two sites were 130 km apart.

Topsoil from both localities was collected as substrata for snail maintenance. Three addi-

TABLE 1. Results of the chemical soil analyses. See text for further explanation of variables.

Soil parameter	Gurnigel, Ca-rich soil	Zastler, Ca-poor soil
Calcium (Ca)*	1,409	99
pH (H_2O)	7.2	5.8
Total nitrogen (%)	0.37	0.30
C/N-ratio	11.5	15.2
Phosphorus (P_2O_5)*	0.2	1.3
Potassium (K_2O)*	19	17
Magnesium (Mg)*	6	17
Copper (Cu)*	0.03	0.24
Iron (Fe)*	0.6	18.0
Manganese (Mn)*	0.3	9.4
Zinc (Zn)*	0.1	1.7

*in mg/100 g dry soil

tional samples from each site were combined for soil analyses: the percentage of nitrogen (N), the content (in mg per 100 g dry soil) of calcium (Ca), phosphorus (P_2O_5), potassium (K_2O), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), the C/N-ratio and the soil pH in water. The soil samples were analysed using standard protocols by the laboratory of F. M. Balzer, Wetter-Amönau, Germany. Characteristics of the two soils are summarized in Table 1. The Ca content represents both the exchangeable calcium and the calcium carbonate content of the soil.

The snails were kept isolated in transparent beakers (8 cm deep, 6.5 cm in diameter) lined with moist soil (approximately 4 cm) outdoors at a shaded place. Fresh lettuce was provided twice per week and at the same time the beakers were cleaned. Snails from both sites were randomly assigned to one of two treatment groups. Half of the snails were raised on soil from their original locality, the other half on soil from the other locality (Gurnigel: 53 individuals on Ca-rich [original] soil, 52 individuals on Ca-poor soil; Zastler: each 51 individuals on Ca-poor [original] and Ca-rich soil).

Within four weeks, all individuals reached sexual maturity as indicated by the formation of a flanged lip at the shell aperture. The snails were marked individually with letters and numbers written on their shells with a waterproof felt-tipped pen on a spot of correction fluid (Tipp-Ex). The animals showed no visible reaction to the marking procedure.

Shell breadth of each snail was measured twice to the nearest 0.1 mm using vernier callipers immediately after being collected as subadults and after shell growth was completed. Relative shell growth of an individual was expressed by the difference between the two measurements divided by the initial size. In adult snails, shell height was also measured and shell volume was calculated using the formula: shell volume = 0.312 x [$(\text{breadth})^2$ x height] – 0.038 (measurements in mm; B. Baur, unpublished data). Shell volume is a more reliable measurement of snail size than weight, because weight depends on the state of hydration and thus is highly variable in terrestrial gastropods.

Sexually mature snails were allowed to mate outdoors. Active snails (individuals with an extended soft body and everted tentacles) from each treatment group were placed in a transparent plastic container, measuring 25 x 18 x 7 cm, lined with moistened paper towelling. When a pair had started to court the snails were transferred to a smaller plastic container (14 x 10 x 7 cm) to allow mating to take place without disturbance from other non-courting snails. Mating trials were initiated in the evening and ran during three nights in June 2002. The period between the end of May and the middle of July is the time of maximum mating activity in subalpine populations of *A. arbustorum* (Baur, 1992a).

We observed the snails‘ courtship behaviour at intervals of 15 min (at night using a torch) following the method described in Baur (1992a) and recorded courtship duration (time interval from courtship initiation to copulation). Observation sessions were terminated either after successful copulation or after 14 h if no snail initiated courtship behaviour. Snails that did not mate were tested again seven days later with a new composition of snails within the same treatment group. Between two trials, snails of each group were maintained as described above.

After copulation, one randomly chosen mating partner (hereafter referred to as focal snail) was kept in a beaker as in the premating phase, but at 19°C with a light:dark cycle of 16:8 h. The other mating partner (hereafter referred to as sperm receiver) was frozen immediately after copulation. To obtain the spermatophore from the focal snail, we dissected out the female reproductive tract of the receiver. We measured the length (*L*) and width (*W*) of the sperm-containing part of each spermatophore to the nearest 0.1 mm using a dissecting microscope. Spermatophore size (in mm^3) was approximated, by the formula ($\pi LW^2/4$), assuming a cylindrical volume. Spermatophores were kept singly in Eppendorf tubes at -30°C until sperm were counted.

The beakers of focal snails were checked for eggs once per week. The eggs of each batch were collected, counted, and kept in a plastic dish (6.5 cm in diameter) lined with moist paper towelling at 19°C to determine hatching success. Newly hatched snails were separated from remaining unhatched eggs to prevent egg cannibalism (Baur, 1992b). In all treatment groups eggs were collected over a period of 60 days following copulation. The length of this period corresponds to approximately one reproductive season of *A. arbustorum* living in the wild (Baur, 1990).

Sperm Counting Procedure

We assessed the number of sperm that a focal snail delivered to its mating partner by counting the number of sperm in the spermatophore transferred. This procedure is described in detail in Locher & Baur (1997). The spermatophore of *A. arbustorum* consists of a hardened secretion which encapsulates the spermatozoa (Hofmann, 1923). We mechanically disrupted the spermatophore in 200 µl PBS-buffer (138.6 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4 \times 2H_2O$ and 1.5 mM KH_2PO_4) using a pair of microscissors. The sperm suspension was homogenized with a set of Gilson pipettes for 5–15 min. To count the sperm, we stained the homogenate for 1–3 h with an equal volume of a gallocyanin-chromium complex, which stains the DNA in the head of the spermatozoa. If spermatozoa still occurred in clusters, we treated the sample overnight with a sonicator (35 kHz). Two subsamples of known volume of the sperm suspension were diluted 1:3 with PBS-buffer and transferred to a Bürker-Türk counting chamber. This counting chamber consists of 16 cells each with a volume of 25 nl. We counted all sperm heads in randomly chosen cells until the total number of sperm heads exceeded 400, and used the average of two subsamples to calculate the total number of sperm in a spermatophore.

Estimate of Sex-Specific Reproductive Allocation

As measures of female reproductive allocation the dry mass of all eggs produced by an individual and the dry mass of the albumen gland were considered. We assessed the dry weight of 48 randomly sampled eggs (three eggs each from eight snails from both populations, equally distributed over the soil types), calculated the mean dry weight of an egg for both populations on each soil type, and multiplied this by the number of eggs produced by each snail. At the end of the experiment, the albumen gland of each focal snail was dissected out of the female reproductive duct. We determined the dry mass of the albumen gland to the nearest 0.1 mg. The dry mass of spermatophores filled with spermatozoa was considered as a measure of male reproductive allocation. We used the relationship between the size of the spermatophores (X = volume of the sperm container in mm^3) and the dry mass of spermatophores filled with spermatozoa (Y in mg)

$$\ln(Y) = 0.670 \times \ln(X) - 0.524$$

to calculate the dry mass of the spermatophores produced by individual snails in these experiments (relationship from Locher & Baur, 2000).

Data Analyses

The StatView program package (SAS Institute, 1998) was used for statistical analyses. Means ± 1 S.E. are given unless otherwise stated. Data which did not fit normal distributions were $\log_{10}$-transformed and frequency data (hatching success) were arcsine-transformed. Differences in shell size and relative growth rate between treatment groups (soil type) and populations were examined using two-way analysis of variance. However, allometric relationships may confound interpretations of differences in observed reproductive output between treatment groups and/or populations. To examine possible differences in reproductive traits analysis of covariance with soil type and population as factors and shell size as covariate was used (ANCOVA, type III model).

RESULTS

Shell Growth and Adult Size

At the beginning of the experiment, subadult snails from the Gurnigel population were smaller than those from the Zastler population (shell breadth: 15.0 ± 0.1 mm vs. 17.9 ± 0.1 mm; $F_{1,120} = 324.24$, $p < 0.0001$). However, subadult *A. arbustorum* from either population kept both on Ca-rich or Ca-poor soil did not differ in shell breadth ($F_{1,120} = 0.0001$, $p = 0.99$).

All snails attained adult size within four weeks. Fully grown snails from the two populations differed in adult size (shell breadth: Gurnigel: 17.3 ± 0.1 mm; Zastler: 19.4 ± 0.1 mm; $F_{1,121} = 124.59$, $p < 0.0001$). In both populations, adult shell size was not affected by the type of soil on which the snails were kept ($F_{1,121} = 1.70$, $p = 0.20$). Relative shell growth differed among snails from the two populations (Gurnigel: 0.155 ± 0.008; Zastler: 0.084 ± 0.006; $F_{1,120} = 53.92$, $p < 0.0001$) and was slightly, but not significantly higher in snails kept on Ca-rich soil than in snails kept on Ca-poor soil ($F_{1,120} = 2.65$, $p = 0.106$).

Mating Propensity

Copulations were observed in 75 (34.9%) out of 215 trials. Snails from the Gurnigel population showed a slightly but not significantly higher mating propensity (40.4%; 40 matings out of 99 trials) than those from the Zastler population (30.2%; 35 matings out of 116 trials; $\chi^2 = 2.46$, df = 1, $p = 0.13$). However, the type of soil affected the mating propensity. Independent of origin, snails kept on Ca-poor soil showed a higher mating propensity than snails kept on Ca-rich soil (43.9% vs. 27.4%; $\chi^2 = 6.41$, df = 1, $p = 0.015$, data from both populations combined). In the Zastler population, a larger proportion of snails kept on Ca-poor soil copulated compared with snails kept on Ca-rich soil (39.6% vs. 22.6%; $\chi^2 = 3.92$, df = 1, $p = 0.048$). Similarly, in the Gurnigel population, there was a tendency that more snails kept on Ca-poor soil copulated than snails kept on Ca-rich soil (50.0% vs. 33.3%; $\chi^2 = 2.79$, df = 1, $p = 0.08$). The time from initiation of courtship to copulation ranged from 6.5 to 24.5 h (mean: 12.3 h, n = 69) and was neither influenced by the type of soil ($F_{1,65} = 0.04$, $p = 0.84$) nor did it differ between the two populations ($F_{1,65} = 0.12$, $p = 0.73$).

Female Reproductive Output

Snails deposited their first egg batch 10 to 39 days after copulation (mean: 13.1 days, n = 69). The time elapsed between copulation and first oviposition was neither influenced by the type of soil ($F_{1,65} = 0.19$, $p = 0.67$) nor did it differ between populations ($F_{1,65} = 0.01$, $p = 0.99$).

Snails from either population did not differ in number of egg batches produced (Table 2). However, the number of egg batches produced was affected by soil type (Table 3). Surprisingly, snails kept on Ca-poor soil deposited more batches than those maintained on Ca-rich soil (Table 2). Furthermore, the number of egg batches laid was influenced by shell size (Table 3). Similarly, the total number of eggs produced by each snail was affected by soil type (Table 3). Snails kept on Ca-poor soil produced more eggs than snails kept of Ca-rich soil (Table 2). The total number of eggs produced was not

TABLE 2. Female and male reproductive traits of *A. arbustorum* from two populations (Gurnigel and Zastler). Snails were kept both on Ca-rich soil (original substratum in Gurnigel) and Ca-poor soil (original substratum in Zastler). Mean values ± 1 S.E. with sample size in parentheses are presented. Results of ANCOVAs are shown (for details of statistical analyses, see Table 3).

Trait	Gurnigel population		Zastler population		Effect of		
	Ca-rich	Ca-poor	Ca-rich	Ca-poor	shell size	population	soil type
Number of egg batches	3.7 ± 0.5 (15)	4.0 ± 0.3 (21)	3.8 ± 0.4 (14)	4.5 ± 0.3 (19)	*	ns	*
Total number of eggs	116 ± 14 (15)	129 ± 12 (21)	139 ± 15 (14)	167 ± 11 (19)	ns	ns	*
Egg mass (mg)	2.45 ± 0.09 (12)	2.18 ± 0.04 (12)	2.34 ± 0.14 (12)	2.44 ± 0.10 (12)	*	**	ns
Number of sperm delivered (x 10^3)	2194 ± 340 (15)	2035 ± 276 (20)	2705 ± 450 (13)	2049 ± 230 (19)	ns	ns	ns
Spermatophore size, (volume of sperm container, mm^3)	1.95 ± 0.21 (15)	1.75 ± 0.15 (20)	2.68 ± 0.28 (14)	2.21 ± 0.19 (19)	*	ns	ns

*p < 0.05, **p < 0.01, ns = not significant affected by shell size (Table 3). In contrast, egg dry mass differed between populations and was significantly related to shell size, but was not affected by soil type (Table 3). Furthermore, the analysis of covariance had a significant interaction term (shell size x population), indicating differing slopes between populations (Table 3). In snails from the Zastler population, egg dry mass was positively related to the shell size of the mother snail (R^2 = 0.52, n = 8, p = 0.0424), whereas in the Gurnigel population no significant relationship was found (R^2 = 0.08, n = 8, p = 0.49).

Hatching success of eggs differed slightly, but not significantly between populations (Gurnigel: 82.3 ± 2.6%, Zastler: 75.3 ± 3.4%; $F_{1,65}$ = 3.62, p = 0.0617), but was not affected by soil type ($F_{1,65}$ = 2.46, p = 0.12).

Female reproductive output, represented by the dry mass of all eggs produced by an individual snail, ranged from 21 to 614 mg (mean: 327 mg, n = 69). Female reproductive output was affected by soil type (Ca-rich soil: 299 ± 25 mg; Ca-poor soil: 347 ± 21 mg; $F_{1,61}$ = 5.14, p = 0.0270), but did not differ between the populations ($F_{1,61}$ = 0.25, p = 0.62) and was not influenced by snail size ($F_{1,61}$ = 0.17, p = 0.68). There was, however, a significant interaction term (shell size x soil type), indicating that shell size influenced female reproductive output on different soils in a different way ($F_{1,61}$ = 4.92, p = 0.0302). The dry weight of the albumen gland, another measure of female reproductive output, ranged from 8.7 to 59.4 mg (mean: 29.5 mg, n = 66). As the dry mass of all eggs, the mass of the albumen gland was affected by soil type ($F_{1,58}$ = 4.45, p = 0.0392), but no effects of population and shell size were found ($F_{1,58}$ = 0.87, p = 0.36 and $F_{1,58}$ = 0.21, p = 0.65).

TABLE 3. Analyses of covariance (ANCOVA) of the relationship between reproductive characters and shell size (volume, log-tranformed) of *A. arbustorum*. Snails from two populations were kept each on two soils (only significant interactions are shown).

Dependent variable	Independent variable, Effects	df	Type III SS	F	p
Number of egg batches (log)	Shell size	1	0.231	6.44	0.0137
	Population	1	0.035	0.97	0.33
	Soil type	1	0.182	5.07	0.0279
	Shell size x soil type	1	0.167	4.66	0.0347
	Error	61			
Total number of eggs	Shell size	1	518.622	0.18	0.68
	Population	1	635.611	0.22	0.64
	Soil type	1	15101.587	5.14	0.0269
	Shell size x soil type	1	14582.744	4.97	0.0295
	Error	61			
Egg mass (log)	Shell size	1	0.018	10.27	0.0125
	Population	1	0.023	13.53	0.0062
	Soil type	1	0.001	0.58	0.47
	Shell size x population	1	0.017	10.02	0.0133
	Error	8			
Number of sperm delivered (log)	Shell size	1	0.173	1.64	0.21
	Population	1	0.051	0.48	0.49
	Soil type	1	0.00003	0.003	0.99
	Error	59			
Spermatophore size, (volume of sperm container, log)	Shell size	1	0.077	5.53	0.0220
	Population	1	0.0001	0.01	0.92
	Soil type	1	0.002	0.13	0.72
	Error	60			

Male Reproductive Output

Spermatophore size, indicated by the volume of sperm container, was neither influenced by soil type nor by population (Table 3). However, spermatophore size was influenced by shell size (Table 3); it was positively correlated with shell volume ($r = 0.42$, $n = 68$, $p = 0.0003$). The number of sperm delivered in a spermatophore ranged from 192,000 to 6,026,000 (mean: 2,204,600, $n = 67$). Sperm number was neither affected by soil type, nor by the origin of the snails (population) and shell size (Tables 2, 3).

The male reproductive output, expressed as dry mass of both spermatozoa and spermatophore, ranged from 0.31 to 1.83 mg (mean: 0.96 mg, $n = 68$). Male reproductive output was affected by shell size ($F_{1,60} = 5.01$, $p = 0.0289$), but not by soil type and population ($F_{1,60} = 0.05$, $p = 0.83$ and $F_{1,60} = 0.11$, $p = 0.74$).

The relative allocation to the male reproductive function, expressed as percentage of the total dry mass devoted to the male function, ranged from 0.1 to 3.2% (mean = 0.4%, $n = 68$). The remaining 96.8 to 99.9% of the dry mass were allocated to the female reproductive output. The relative allocation to the male reproductive function was neither influenced by soil type, population nor by shell size (in all cases $p > 0.20$).

DISCUSSION

The present study showed that the type of soil can affect the mating propensity and female reproductive output in a simultaneous hermaphrodite land snail. Mating propensity and other life-history traits including adult shell size, shell growth, and egg size were also influenced by the snails' origin, and still others (egg size, number of egg batches produced and spermatophore size) were affected by shell size. Effects of soil type on reproductive traits have so far received little attention in terrestrial gastropods. The breeding behaviour of *Cernuella virgata* (da Costa, 1778) was affected both by soil type and soil moisture content, whereas the total number of eggs laid and the tendency to lay the first egg were influenced by soil type (Carne-Cavagnaro et al., 2006). Juveniles of *Cornu aspersum* (Müller, 1774) from different sites exhibited differential growth rates and Ca-allocation strategies (Beeby & Richmond, 2007). Land snails may be able to assess the Ca concentration in their food plants and in the soil (Chevalier et al., 2003) and may actively balance their diet to optimise the Ca intake (Iglesias & Castillejo, 1999). Soil constitutes a key component of the snails' diet in natural populations. In *C. aspersum*, hatchlings which fed on soil outgrew other siblings, indicating the importance of certain soil characteristics and of exchangeable Ca (Gomot et al., 1989).

In a laboratory experiment, the Ca concentration did not affect shell growth and adult size in subadult individuals of *A. arbustorum* reared on agar-based diets with different levels of Ca (Wacker & Baur, 2004). Snails reared on intermediate- and low-calcium diets increased their consumption rates, but despite compensatory feeding, these snails were unable to take up the amount of Ca required for metabolism and shell growth and thus had a higher mortality.

The Ca-provision to the eggs represents a major cost to the parent. Among gastropods, a variety of strategies are used to ensure sufficient Ca to the eggs (Tompa, 1980; Baur, 1994a). Ca is used for the calcification of the embryonic shell and for the deposition of Ca reserves which differentiate during the embryonic life (Fournié & Chétail, 1984). The Ca requirements for embryonic life explain the considerable amount of Ca that the adult gastropod looses during the oviposition period. For example, *Anguispira alternata* (Say, 1816) mobilizes 10–25 mg Ca for one egg batch in less than a day (Tompa, 1975).

In several species of terrestrial gastropods, a significant amount of Ca is embedded in the egg shell. *Arianta arbustorum* has partly calcified eggs with discrete crystals of calcium carbonate in a jelly matrix. Compared with species that produce similar eggs, the Ca concentration in *A. arbustorum* eggs may range from 5 to 8% of their dry mass (cf. Tompa, 1976). Thus, snails kept on Ca-poor soil may have invested 17–28 mg Ca into the eggs produced in the course of the experiment, those kept on Ca-rich soil 15–24 mg Ca. The Ca-content of lettuce varies between 300 and 500 mg/kg (Chevalier et al., 2003). Individual *A. arbustorum,* kept under similar experimental conditions as in the present study, consumed a leaf area of 32–50 cm^2 lettuce per week, which corresponds to a Ca-uptake of 0.26 to 0.68 mg per week (Locher & Baur, 2002). The assimilation efficiency of consumed lettuce averages 97% in *A. arbustorum* (dry mass; Abdel-Rehim, 1987). Considering the entire experimental period of 88 days, this resulted in a Ca-uptake of 3.3–8.5 mg, indicating that a considerable amount of Ca invested in egg production was actually obtained from the lettuce consumed. Thus, snails kept on Ca-poor soil could partly compensate the Ca-deficiency in the soil by consuming more lettuce. It should be noted, however, that the experimental conditions in our study did not represent the natural situation, because plants growing on Ca-poor soils usually have a low Ca-content. The same might be true for decaying plant material and leaf litter on Ca-poor soil.

Scarcity of Ca may result in thinner and more brittle shells (Voelker, 1959), rendering the snails less fit to protect the soft body properly against desiccation, physical damage and invertebrate predators. *Arianta arbustorum* shows a tolerance to acidic soils with low Ca availability (Kerney & Cameron, 1979). In the wild, however, adult *A. arbustorum* with more robust shells were found at the Ca-rich site at Gurnigel than at the Ca-poor site at Zastler (B. Baur, unpubl. data).

Because snails require substantial amounts of Ca for reproduction (Crowell, 1973; Wäreborn, 1979), growth (Gomot et al., 1989; Ireland, 1991), shell production, and metabolism (Fournié & Chétail, 1984), Ca availability is a key determinant of habitat quality in molluscs. In fact, greater abundance, species richness, and biomass of snails are generally observed on soils rich in Ca compared with soils poor in Ca (Boycott, 1934; Wäreborn, 1992; Hotopp, 2002; Ondina et al., 2004). Ca addition to mitigate effects of acid deposition resulted in a significant increase in snail abundance (Gärdenfors, 1992; Skeldon et al., 2007), whereas slugs did not show a similar pattern. However, processes other than acid deposition also influence Ca cycling and availability in soils. For example, wood succession resulting in a shift in the dominant tree species can change the foliar Ca concentration.

In suboptimal habitats where conditions are outside the range to which most snail individuals are adapted, some cost, expressed either as a reduction in growth, survival, or reproduction, may occur. However, apart from Ca availability, other soil parameters, such as soil physical properties (soil texture, moisture retention and chemistry) and availabilities of nutrients such as aluminium, nitrogen and magnesium, may influence the reproductive output of *A. arbustorum*. Soil type seems to be a key factor in the reproductive biology of *A. arbustorum* and most probably in other land snail species. However, it remains unclear which soil characteristics are important.

In our study, the total number of eggs produced was larger in snails kept on Ca-poor soil than in individuals maintained on Ca-rich soil. There are different possible explanations for this surprising finding: (1) the Ca-poor soil used in the experiment still contained enough Ca to allow egg production; (2) the snails ingested Ca from the lettuce offered and with compensatory feeding they partly counter-balanced the Ca deficiency; (3) the Ca-rich soil could contain minerals or (unknown) substances that disturbed reproduction in *A. arbustorum*; (4) the Ca-poor soil had other essential constituents for successful reproduction that were in short supply in the Ca-rich soil; (5) snails living under environmental stressing conditions (i.e., on Ca-poor soils) put more resources into reproduction in their first breeding but have a shorter lifespan than snails in more favourable conditions. The range of Ca content in different soil types is much wider in nature than in the two soils used in this experiment. Our Ca-poor soil still contained a low level of exchangeable Ca (Table 1). The hypothesis of compensatory feeding cannot be tested because the amount of lettuce consumed by each snail was not recorded. However, the albumen gland of snails kept on Ca-poor soil was larger than that of snails maintained on Ca-rich soil. This indicates that the former group ingested more food. In stylommatophoran gastropods, the albumen gland synthesizes the perivitelline fluid to be added to eggs for provisioning the developing embryo (Gomez, 2001). The size of this gland determines the maximum number of eggs that can be produced at any one time (Tompa, 1984). Most probably, snails kept on Ca-rich soil consumed for some unknown reasons less lettuce, which resulted both in a decreased mating propensity and reproductive output. Hatching success of eggs produced by snails kept on either soil type did not differ, suggesting that there was no difference in egg quality between the two treatment groups. At high concentrations, zinc, copper, lead and cadmium all negatively affect reproduction in *C. aspersum* (Laskowski & Hopkin, 1996). At low concentrations, however, no effect of these metals was recorded (Gomot, 1997). In contrast, other soil constituents may positively affect snail reproduction. For example, the Ca-poor soil contained three times more magnesium than the Ca-rich soil (Table 1). Unfortunately, besides Ca relatively little is known on the beneficial value of various soil constituents for land snail reproduction (Speiser, 2001). There may also be a trade-off between reproductive output and survival. Snails living in environmentally stressing conditions may allocate more resources into reproduction in the first breeding season but may die earlier than those living in more favourable conditions. This hypothesis could be tested by maintaining snails over two or more years under the experimental conditions of the present study. We assume that a combination of different factors contributed to the unexpected result.

The present study examined to our knowledge for the first time soil-type related effects on male reproductive output. However, neither the number of sperm delivered nor spermatophore size differed between the two snail groups kept on different soils. Of the total reproductive output (expressed as dry mass), only 0.1–3.2% were devoted to the male function in form of sperm and spermatophore, while the remaining 96.8–99.9% were allocated to the female function in form of eggs. These values are very similar to previous estimates of male and female reproductive allocation in *A. arbustorum* (Locher & Baur, 2000, 2002).

In our study, several life-history traits were affected by the origin of the snails and/or their adult size. Size-related fecundity has been found in a variety of gastropods as well as in other invertebrates (Baur, 1998). In many land snail species, both clutch size and egg size are positively correlated with shell size (Heller, 2001). In the present study, the number of egg batches deposited, egg mass and spermatophore size were related to adult size in *A. arbustorum*.

In land snails, limited dispersal capacity and restricted habitat requirements may enhance the process of adaptation to local conditions. Genetic differentiation between populations of terrestrial gastropods has been demonstrated in several species (e.g., Jones et al., 1977; Clarke et al., 1978; Fearnley, 1996; Beeby & Richmond, 2001, 2007). The adult size of *A. arbustorum* decreases with increasing altitude in the Alps, whereas shell colour varies with the type of habitat (Burla & Stahel, 1983; Baur, 1984; Gosteli & Burla, 1993). Snails from different populations exhibit differences in resting site preference and geotactic response (Baur, 1986; Baur & Gosteli, 1986), as well as in such reproductive characters as clutch size and egg size (Baur & Raboud, 1988). Snails from the two populations examined in the present study differed in adult size, relative shell growth, mating propensity and egg size.

In conclusion, the present study showed that soil type can affect reproductive traits in *A. arbustorum*. However, some reproductive traits were also influenced by the origin of the snails and by shell size emphasizing the importance of proper design and replication of life-history studies in gastropods.

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BENTHOCTOPUS RIGBYAE, N. SP., A NEW SPECIES OF CEPHALOPOD (OCTOPODA; INCIRRATA) FROM NEAR THE ANTARCTIC PENINSULA

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ABSTRACT

Among the many octopods collected during recent Antarctic trawling surveys were 93 specimens of an undescribed octopod with biserial suckers. Although most similar in appearance to the sub-Antarctic *Benthoctopus levis* (Hoyle 1885), these octopods differed in several morphological characters, including arm length, web depth, and details of the hectocotylus. Furthermore, molecular evidence supports the separation of the present material from known species. We therefore describe a new species named ***Benthoctopus rigbyae***. These octopods attain mantle lengths of at least 105 mm (400 mm total length), and they are common at depths of 250–600 m throughout the South Shetland Island chain off the Antarctic Peninsula. We present some information on the biology of this species.

Keywords: deep-sea, Southern Ocean, Octopodidae, Antarctica.

INTRODUCTION

Incirrate octopods are common members of the Antarctic marine benthos (Dell, 1972; Allcock et al., 2001). Most of the octopods collected in Antarctic samples belong to genera with uniserial suckers, such as *Pareledone, Megaleledone,* and their relatives (e.g., Allcock, 2005; Daly & Rodhouse, 1994; Kuehl, 1988; Kubodera & Okutani, 1994; Lu & Stranks, 1994; Taki, 1961). During recent trawling cruises in the vicinity of the Antarctic Peninsula (Piatkowski et al., 1998, 2003; Barratt & Jorgensen, 2008), we collected 93 specimens of an octopod possessing biserial suckers, but lacking an ink sac. These octopods, which came from depths of about 200–600 m, are similar to *Benthoctopus levis* (Hoyle, 1885). The type locality of *B. levis* is at a depth of about 140 m, near Heard Island, a sub-Antarctic Island in the Indian Ocean. The peninsula specimens differ from *B. levis* in relative arm length, web depth, and hectocotylus details. We therefore consider the peninsula specimens to be members of a newly discovered species of *Benthoctopus*, which we here name ***B. rigbyae***.

MATERIALS AND METHODS

R/V *Polarstern*'s cruises ANT XIV/2 (November–December 1996), ANT XIX/3 (January–February 2002), and ANT XXIII/8 (December 2006–January 2007) focused on the South Shetland Islands northwest of the Antarctic Peninsula. One research component of each cruise was a fisheries survey using commercial-sized bottom trawls (Piatkowski et al., 1998, 2003). Sampling was conducted on ANT XIV/2 at 40 stations around Elephant Island based on a stratified-random survey design in depths of 100–500 m. Two transects of stations northwest of King George Island at depths of 400, 600, and 800 m were also sampled with the same gear. A similar survey during ANT XIX/3 trawled at 49 stations around Elephant Island, 21 stations from 100–500 m depth in the southern South Shetlands, and five stations on the shelf north of Joinville Island. ANT XXIII/8 sampled 51 bottom-trawl stations around Elephant Island and another 38 stations across the Bransfield Straits close to the Peninsula, around Joinville Island and in the western Weddell Sea. Additionally, 23 samples were

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TABLE 1. Criteria used for male and female maturity stages.

	Male	Female
Stage 1	Sex undeterminable	Sex undeterminable
Stage 2	Testes developing or developed, but no spermatophores developing	Ovary small and round, eggs tiny
Stage 3	Spermatophores developing but none are mature	Eggs begin to enlarge
Stage 4	Few mature spermatophores present in Needham's sac	Ovaries swollen and oviducal glands enlarged
Stage 5	Four or more mature spermatophores present in Needham's sac and terminal organ	Ovaries completely distended with many mature eggs
Stage 6	Needham's sac swollen but empty	Ovaries swollen but empty, oviducal glands enlarged

collected with an Agassiz beam-trawl on ANT XIV/2, nine with a smaller Agassiz trawl on ANT XIX/3, and 15 on ANT XXIII/8, at various depths between 100–5,200 m. Details of all stations, including those that did not yield *Benthoctopus*, can be found in the cruise reports (Arntz & Brey, 1997; Kattner et al., 1998; Barratt & Jorgensen, 2008). Details of stations yielding *Benthoctopus* are provided in Figure 8.

All cephalopods were retrieved from all samples. Station locations and distribution of these cephalopods are presented by Piatkowski et al. (1998, 2003) and Barratt & Jorgensen (2008). Dorsal mantle length (ML) and total length were measured and sex and maturity stage determined according to set criteria (Table 1) for all specimens.

Under material examined, we list stage 1 as juvenile, stages 2–3 as immature, stages 4–5 as mature, and stage 6 as spent. Additionally for 15 males and 12 females representing the full range of size and maturity encountered, the following measurements and counts (following Roper & Voss, 1983) were recorded prior to fixation: measurements (Tables 2, 3) – ventral mantle length, mantle width, head width, eye length, width of pallial aperture, full funnel length, free funnel length, web formula, depth of deepest web segment, arm width, length of each arm, sucker diameter; counts (Table 4) – numbers of suckers on left (males and females) and right (males only) ventrolateral arms, number of suckers on the longest arm, number of inner and outer gill lamellae. The lengths of the calamus and ligula were recorded for males and, for six mature males, the length and width of a mature spermatophore as well. For six mature females, the length and width of the largest ovarian egg was measured.

Some specimens were dissected to examine internal anatomy prior to fixation. Dissections emphasized male and female reproductive anatomy and comparative anatomy of digestive tracts. Eleven females and 24 males were weighed to examine the relationship between length and weight. Stomach contents were determined for a few specimens, selected arbitrarily.

Molecular Analyses

Tissue samples were taken from two *Benthoctopus* specimens from the Antarctic Peninsula (NMSZ 2002037.032 and USNM 1021054) and preserved in 70% ethanol. Tissue samples were also preserved in 70% ethanol from a specimen of *Benthoctopus* sp. captured in the eastern Weddell Sea and from two specimens of *Benthoctopus levis* captured around Heard Island. We also obtained a beak taken from a *Benthoctopus* specimen presumed to be *B. thielei* Robson, 1932, captured off Kerguelen Island, and extracted DNA from this. Although DNA has previously been extracted from beach-washed *Spirula* shells (Strugnell et al., 2006), we believe that this is the first published report of DNA from a cephalopod beak.

The DNA extraction protocol followed that given in Allcock et al. (2006). Primers for three mitochondrial genes (12S rDNA, 16S rDNA, COI) were taken from the literature (Simon et al., 1990, 1991; Folmer et al., 1994), with the 12S rDNA and COI primers modified slightly to match cephalopod sequences on GenBank. Polymerase chain reactions (PCR) were carried out in 25 ml volumes. Thermal cycling conditions consisted of a denaturation step at 94°C for 2 min, followed by 35 cycles of 94°C

TABLE 2. Measurements (mm) of type specimens of ***Benthoctopus rigbyae*** n. sp. as archived at NMNH. Dorsal mantle length (prior to fixation) was measured on freshly collected specimens onboard ship.

	Holotype	Paratypes	
Sex	male	female	female
Total length	229	259	243
Dorsal mantle length (prior to fixation)	64	55	52
Dorsal mantle length (preserved)	49	47	42
Ventral mantle length	43	46	38
Mantle width	37	45	33
Head width	23	28	25
Eye diameter	9	8	6
Pallial aperture width	25	29	26
Full funnel length	16	17	14
Free funnel length	12	13	10
Deepest web segment depth	26	47	30
Arm width (longest arm)	5	4	5
Left dorsal arm length	176	200	168
Left dorsolateral arm length	175	202	169
Left ventrolateral arm length	147	201	180
Left ventral arm length	164	196	166
Right dorsal arm length	179	200	165
Right dorsolateral arm length	156	182	179
Right ventrolateral arm length	127	189	167
Right ventral arm length	166	183	172
Diameter of largest sucker	5	6	5
Calamus length	6		
Ligula length	17		
Web depth, segment A	21	47	29
Web depth, segment B	26	33	28
Web depth, segment C	26	29	damaged
Web depth, segment D	25	29	30
Web depth, segment E	25	16	24

for 40 s, 50°C for 40 s, and 72°C for 90 s. A final extension step of 72°C for 10 min was added in each case. Annealing temperatures varied according to the primers used and are available from the authors on request. Amplified products were purified using the QiaGen PCR purification kit following the manufacturer's instructions. Purified PCR products were commercially sequenced by Macrogen Inc. in both directions using the same primers used for PCR amplification. Sequences for *Enteroctopus dofleini* (Wülker, 1910), *Benthoctopus normani* (Massy, 1907), *Benthoctopus eureka* (Robson, 1932), and *Benthoctopus johnsoniana* Allcock et al., 2006, collected from the northeastern Pacific, the Falkland Islands and the northeastern Atlantic respectively were available from previous research (Allcock et al., 2006).

DNA sequences were compiled and aligned by eye in Se-Al v2.0a11 Carbon (Rambaut 2002). The sequence data for each gene were concatenated into a single data set. Of the 1,612 characters used in the analysis, 177 (11%) were found to be variable.

PAUP v4.0b10 (Swofford, 1998) was used to perform full heuristic searches. The phylogenetic tree is rooted using *Enteroctopus dofleini*, as previous phylogenetic studies using

TABLE 3. Measurements (mm) of ***Benthoctopus rigbyae*** n. sp. Abbreviations: min – minimum; max – maximum; s.d. – standard deviation; c.v. – coefficient of variation; N – number of octopods measured. Measurements were recorded at sea on freshly collected specimens. Total length and dorsal mantle length were recorded when specimens condition was adeqaute whereas other measurements were recorded on a subset of specimens when time permitted. Spermatophore and egg measurements are from the largest of each from N individuals rather than multiple spermatophores or eggs from a single individual.

	min	max	median	mean	s.d.	c.v.	N
Total length	100	400	266	258	68	0.26	78
Dorsal mantle length	18	105	60	60	18	0.31	79
Ventral mantle length	28	85	60	58	15	0.26	27
Mantle width	28	80	65	59	14	0.24	27
Head width	21	49	40	39	7	0.18	28
Eye diameter	11	28	18	19	4	0.20	28
Pallial aperture width	18	50	35	35	7	0.20	27
Full funnel length	15	36	27	27	6	0.21	28
Free funnel length	12	30	19	19	5	0.26	28
Deepest web segment depth	13	55	46	45	9	0.21	24
Arm width (largest arm)	6	20	14	14	3	0.21	28
Left dorsal arm length	128	330	230	219	55	0.25	23
Left dorsolateral arm length	137	285	223	213	45	0.21	22
Left ventrolateral arm length	127	320	223	216	50	0.23	26
Left ventral arm length	124	300	223	217	49	0.23	27
Right dorsal arm length	132	320	215	221	51	0.23	25
Right dorsolateral arm length	130	320	225	220	52	0.24	25
Right ventrolateral arm length	105	280	185	180	43	0.24	26
Right ventral arm length	112	300	221	220	51	0.23	24
Diameter of largest sucker	3	7	5	5	1	0.23	28
Calamus length	4	12	9	8	2	0.25	15
Ligula length	8	26	18	17	5	0.26	15
Spermatophore length	42	104	88	82	23	0.29	6
Spermatophore width	3	3	3	3	0	–	5
Length of largest egg	13	24	18	17	4	0.24	6
Width of largest egg	3	8	7	6	2	0.32	6
Web depth, segment A	8	55	42	40	12	0.31	15
Web depth, segment B	13	55	38	38	13	0.33	15
Web depth, segment C	9	55	44	41	13	0.31	16
Web depth, segment D	11	55	45	42	11	0.26	16
Web depth, segment E	11	50	32	33	11	0.35	15

a wide range of octopodiform species have confirmed that *Enteroctopus* is the sister taxon to *Benthoctopus* (Strugnell et al., 2005) and subsequently that *Enteroctopus* is a suitable outgroup to the genus *Benthoctopus* (Allcock et al., 2006). Starting trees were generated by neighbour joining (NJ) (Saitou & Nei, 1987). A GTR (Γ + I) likelihood model incorporating rate heterogeneity (four rate categories) was used. Branch swapping was performed using TBR (tree-bisection-reconnection). Parameters were then re-estimated and finally branch swapping was performed using NNI (nearest-neighbour interchange). Substitution model parameter values were A = 0.34, C = 0.12, G = 0.15, T = 0.39, $A \leftrightarrow C = 1.48*e^{8}$, $A \leftrightarrow G = 1.53*e^{9}$, $A \leftrightarrow T = 2.80*e^{8}$, $C \leftrightarrow G = 5.55*e^{-11}$, $C \leftrightarrow T = 2.50*e^{9}$ $G \leftrightarrow T = 1.00$, I = 0.81, $\Gamma = 300$. ML bootstrap values of

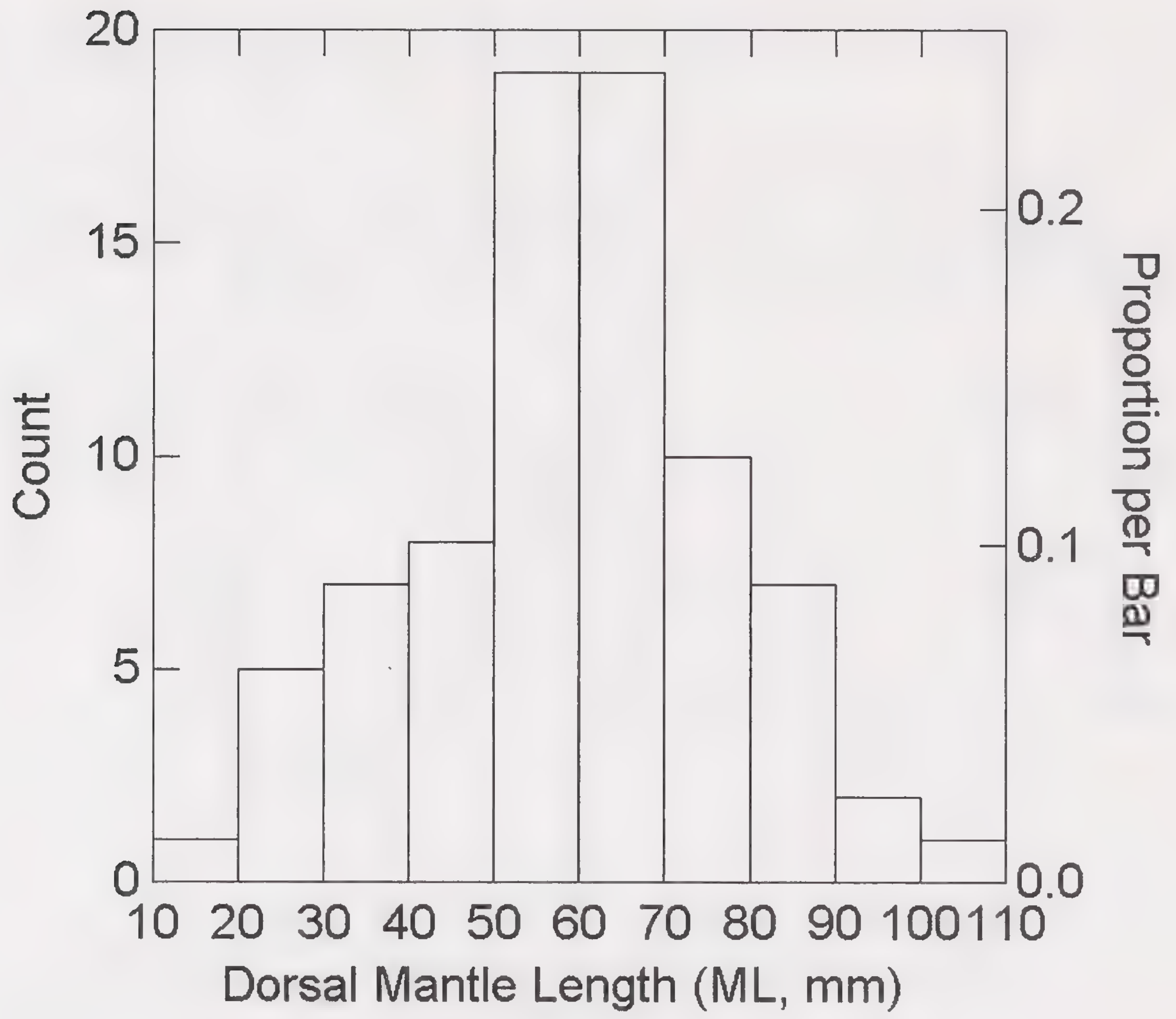

FIG. 1. Size-frequency histogram for 84 specimens of ***Benthoctopus rigbyae*** n. sp. examined in this study.

clade support were generated using the above parameters using 1,000 replicates.

MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) was used to calculate marginal posterior probabilities using the GTR + I + Γ model of nucleotide substitution for each partition. Model parameter values were treated as unknown and were estimated in each analysis. Random starting trees were used and analyses were run 1 million generations, sampling the Markov chain every 100 generations. The analysis was performed twice, in each case starting from a different random tree to ensure the analyses were not trapped in local optima. Stationarity was deemed to be reached when the average standard deviation of split frequencies, shown in MrBayes 3.1.2 was less than 0.01 (Ronquist & Huelsenbeck, 2003).

The program Tracer v1.3 (Rambaut & Drummond, 2003) was used to determine the correct "burn-in" for the analysis (i.e., the number of initial generations that must be discarded before stationarity is reached).

RESULTS

The 93 *Benthoctopus* specimens from the Antarctic Peninsula ranged in ML from 18–105 mm. Size-frequency distribution was unimodal with the strong mode from 50–70 mm ML (Fig. 1). The relationship between ML and total length was essentially linear (Fig. 2). Sex ratio was 1.6, male dominant.

Molecular Analyses

Sequences generated in this study are available from GenBank under accession numbers FJ428003–FJ428015.

The sequences of the two ***Benthoctopus rigbyae*** n. sp. individuals from stations 61/048-1 and 42/003 on the Antarctic Peninsula (Fig 3) were identical (PP = 100, BS = 98). These specimens form a monophyletic group with *Benthoctopus* sp. from station 39/014 in the Weddell Sea, which is highly supported by Bayesian posterior probabilities (PP) (PP = 100) and maximum likelihood bootstrap (BS) values (BS = 92). The sequences of *Benthoctopus* sp. from sta 39/014 in the eastern Weddell Sea (Fig. 1) differ from those of ***Benthoctopus rigbyae*** n. sp. by 0.7% (4 base pairs) for the gene 16S rDNA and 2.7% (18 base pairs) for the gene COI.

The sequences of the two *Benthoctopus levis* individuals, captured from Heard Island, were almost identical (differing by only 0.15% (1 base pair) in the gene COI and 0.18% (1 base pair) in the gene 16S rDNA and grouped together in the tree (PP = 100, BS = 100). *Benthoctopus levis* is the sister taxa to *Benthoctopus thielei* (PP = 62, BS = 62) from Kerguelen Island. The clade containing *Benthoctopus levis* and *Benthoctopus thielei* forms a sister taxa relationship to the clade containing ***Benthoctopus rigbyae*** n. sp. and *Benthoctopus* sp. from the eastern Weddell Sea. This relationship is not highly supported however.

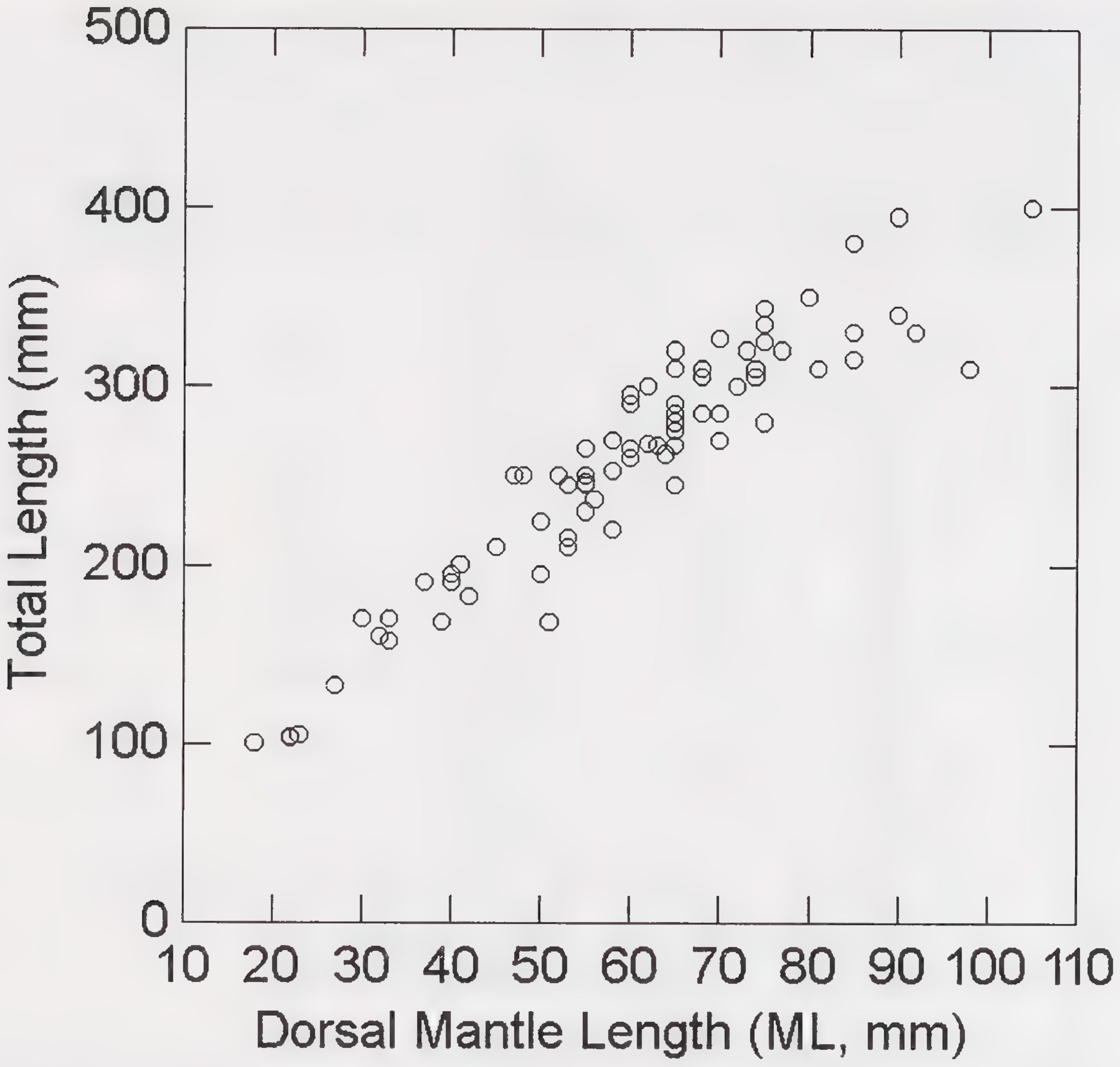

FIG. 2. Relationship between dorsal mantle length and total length of ***Benthoctopus rigbyae*** n. sp.

A sister taxa relationship is present between the clade containing *Benthoctopus* from the Southern Ocean (***Benthoctopus rigbyae*** n. sp., *Benthoctopus* sp. from the eastern Weddell Sea, *B. levis* and *B. thielei*) and *B. johnsoniana*. (PP = 98, BS = 84). *Benthoctopus normani* is basal within the *Benthoctopus* species included in this phylogenetic study.

Systematics

Benthoctopus rigbyae, n. sp.

Holotype: *Polarstern* ANT XIV/2 sta. 42/022, mature male, 64 mm ML, USNM 1117765.

Paratypes: *Polarstern* ANT XIV/2 sta. 42/022, 2 mature females, 55 and 52 mm ML, USNM 1117766.

Other material examined: *Polarstern* ANT XIV/2: Sta. 42/003, 2 immature males, 27 and 48 mm ML, 2 immature females, 24 and 30 mm ML, USNM 1021054; sta. 42/014, 1 immature male, 58 mm ML and 1 mature female, 90 mm ML, USNM 1020987; sta. 42/017, 1 spent? female, 58 mm ML; sta. 42/020, 1 immature male, 39 mm ML; sta. 42/021, 1 mature male, 74 mm ML, 1 immature male, 51 mm ML, 1 juvenile, 18 mm ML; sta. 42/022, 3 immature males, 22–37 mm ML; sta. 42/023, 1 male, mantle and viscera missing, USNM 1021986; sta. 42/029, 2 mature females, 47 and 81 mm ML, 1 male, mantle and viscera missing; sta. 42/036, 2 mature males, 73 and 85 mm ML, 1 mature female, 66 mm ML, 1 immature male, 45 mm ML, 1 immature female, 32 mm ML; sta. 42/040, 5 mature males, 65–75 mm ML, 3 mature females, 48-90 mm ML, 1 immature male, 40 mm ML; sta. 42/041, 1 mature male, 53 mm. ML; sta. 42/043, 1 mature female, 65 mm ML; sta. 42/047, 1 mature female, 50 mm ML; sta. 42/080, 1 mature male, 63 mm ML. *Polarstern* ANT XIX/3: Sta. 61/044-1, 1 mature female, 70 mm ML, NMSZ 2002037.030; sta. 61/045-1, 1 mature male, 98 mm ML, NMSZ 2002037.031; sta. 61/048-1, 1 immature male, 42 mm ML, NMSZ 2002037.032; sta. 61/049-1, 2 immature females, 70-75 mm ML, NMSZ 2002037.033; sta. 61/059-1, 9 mature males, 55-85 mm. ML, 5 immature males, 40–65 mm ML, 2 mature females, each 55 mm ML, 2 immature females, 45-60 mm ML, 1 juvenile female, 30 mm ML, 5 specimens preserved, NMSZ 2002037.034; sta. 61/060-1, 1 immature female, 41 mm. ML; sta. 61/062-1, 1 mature male, 65 mm ML; sta. 61/064-1, 3 mature males, 75–92 mm ML, 1 immature male, 62 mm ML, 1 juvenile male, 33 mm ML, 3 immature females, 56–68 mm ML; sta. 61/101-1, 1 immature male, 60 mm ML; sta. 61/103-1, 2 mature males, 72–85 mm ML, 1 juvenile male, 27 mm ML, 2 mature females,

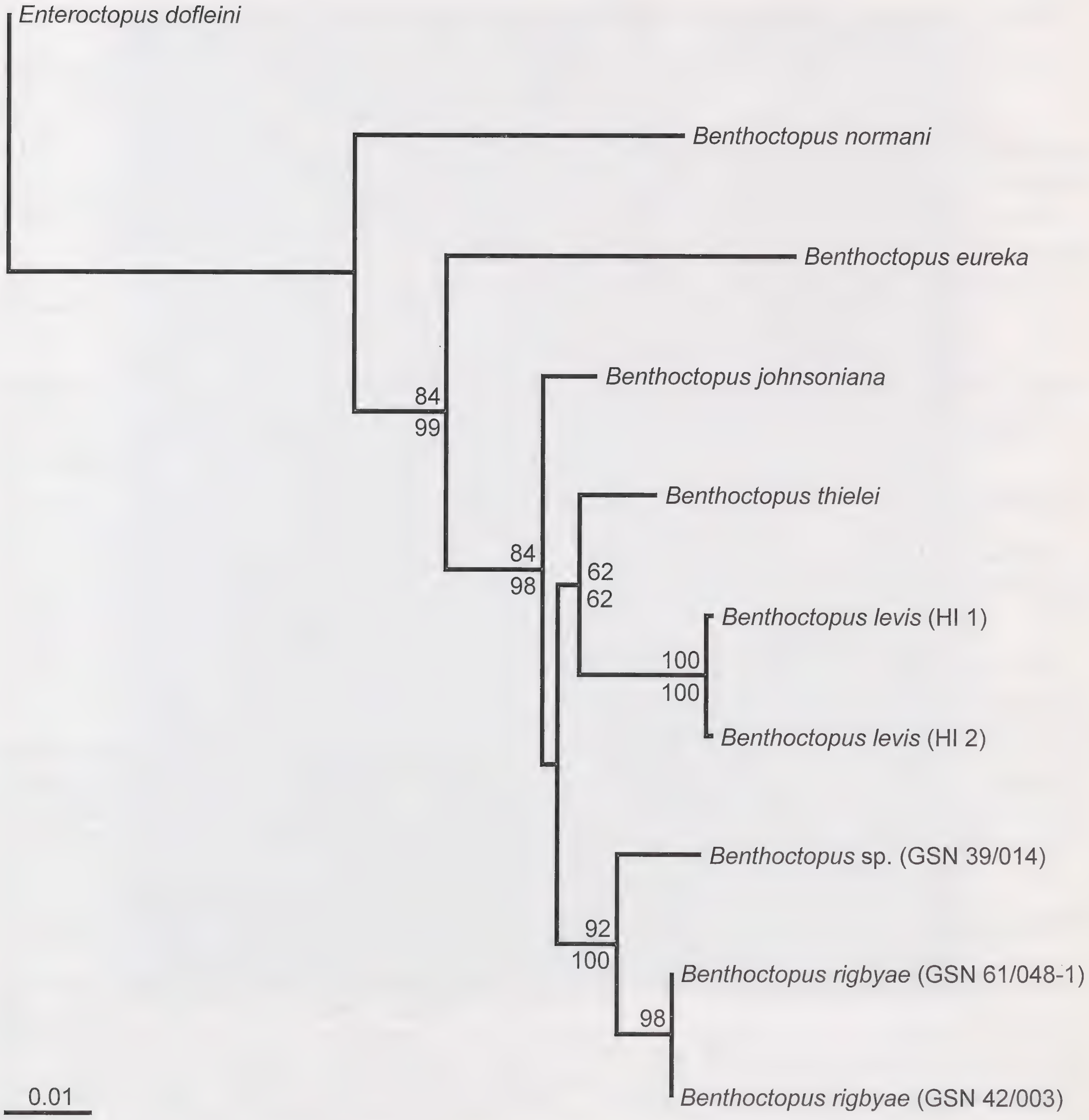
Enteroctopus dofleini
Benthoctopus normani
Benthoctopus eureka
Benthoctopus johnsoniana
Benthoctopus thielei
Benthoctopus levis (HI 1)
Benthoctopus levis (HI 2)
Benthoctopus sp. (GSN 39/014)
Benthoctopus rigbyae (GSN 61/048-1)
Benthoctopus rigbyae (GSN 42/003)
84
99
84
98
62
62
100
100
92
100
98
0.01

FIG. 3. Maximum likelihood tree depicting the phylogenetic relationship of seven species (ten individuals) of Octopoda. The analysis employed three mitochondrial sequences (12S rDNA, 16S rDNA, COI) concatenated. Bayesian support values are indicated below the nodes, maximum likelihood bootstrap values are indicated above the nodes.

80–105 mm ML, 1 immature female, 60 mm ML; sta. 61/107-1, 1 juvenile male, 23 mm ML; sta. 61/109-1, 1 mature female, 53 mm ML, 1 immature female, 53 mm ML; sta. 61/111-1, 1 juvenile male, 22 mm ML. *Polarstern* ANT XXIII/8: sta. 69/609-1, 1 juvenile, 24 mm ML; sta. 69/616-1, 1 immature female, 41 mm ML; sta. 69/652-1, 1 immature female, 20 mm ML; sta. 69/654-6, 1 immature female, 32 mm ML; sta. 69/661-2, 1 immature female, 57 mm ML; sta. 69/662-1, 1 mature male, 61 mm ML; sta. 69/663-1, 1 immature female, 47 mm ML, sta. 69/664-1, 2 immature female, 34–49 mm ML.

Diagnosis

Antarctic octopod with biserial suckers and W-shaped funnel organ, lacking an ink sac. Arms long, 72–94% of total length, with shallow webs approximately 20% of longest arm

TABLE 4. Counts from specimens of ***Benthoctopus rigbyae*** n. sp. See Table 3 for abbreviations.

	min	median	mean	s.d.	c.v.	max	N
Suckers on hectocotylized arm	61	66	67	4	0.06	78	15
Suckers on left ventrolateral arm	113	148	144	14	0.1	165	25
Suckers on longest arm	124	146	144	11	0.08	164	24
Inner gill lamellae	5	7	7	0.7	0.12	8	27
Outer gill lamellae	6	6	7	0.6	0.1	8	27

length. Hectocotylized arm with 60–80 suckers, 56–75% length of longest arm. Ligula length 6–16% of hectocotylized arm length.

Description

Measurement, counts, and indices and their associated statistics are summarized in Tables 3–5.

General Appearance of Live/Fresh Animal (Fig. 4): A moderately large octopod, to at least 105 mm ML, 400 mm TL. Chromatophores very tiny. Overall color light brownish orange, grading to lighter color ventrally and orally but darker at bases of suckers on some animals. Some animals showed a blotchy pattern on the mantle and web, but no changes in color pattern were noted during observation of live animals. Skin smooth, without papillae or superocular cirri. Mantle with obvious dorsal hump, no distinct lateral ridge or keel, although some animals exhibited a band of raised skin laterally on mantle. Arms long. Web formula variable; web shallow, extends along ventral arm to tip and along dorsal arm, attaching to aboral arm surface approximately 1/3–1/2 arm length from proximal. Eyes prominent, dark, with bluish sheen. Mantle muscular.

External Morphology (Fig. 5): Head narrower (approximately 67%) than mantle, head width about 63% ML. Mantle width only slightly less than ML. Arms often regenerating but very long,

TABLE 5. Indices from specimens of ***Benthoctopus rigbyae*** n. sp. See Table 3 for abbreviations.

	min	median	mean	s.d.	c.v.	max	N
Dorsal mantle length / Total length	0.18	0.23	0.23	0.03	0.11	0.32	78
Ventral mantle length / Dorsal mantle length	0.77	0.91	0.91	0.08	0.08	1.05	27
Mantle width / Dorsal mantle length	0.72	0.93	0.93	0.09	0.09	1.15	27
Head width / Dorsal mantle length	0.46	0.61	0.62	0.08	0.13	0.79	27
Head width / Mantle width	0.53	0.64	0.67	0.08	0.12	0.84	27
Eye diameter / Dorsal mantle length	0.22	0.29	0.30	0.04	0.14	0.38	27
Full funnel length / Dorsal mantle length	0.36	0.44	0.43	0.05	0.12	0.54	27
Free funnel length / Dorsal mantle length	0.21	0.31	0.30	0.05	0.15	0.41	27
Deepest web depth / Dorsal mantle length	0.41	0.71	0.72	0.15	0.20	0.98	23
Deepest web depth / Longest arm length	0.09	0.18	0.19	0.03	0.18	0.25	24
Calamus length / Dorsal mantle length	0.09	0.12	0.13	0.02	0.14	0.15	14
Calamus length / Hectocotylized arm length	0.03	0.05	0.05	0.01	0.24	0.08	15
Ligula length / Dorsal mantle length	0.18	0.27	0.26	0.03	0.13	0.30	14
Ligula length / Hectocotylized arm length	0.06	0.10	0.11	0.03	0.25	0.16	15
Longest arm length / Dorsal mantle length	2.50	3.77	3.75	0.63	0.17	4.89	27
Longest arm length / Total length	0.72	0.83	0.83	0.07	0.08	0.94	25
Hectocotylized arm length/Opposite arm length	0.56	0.76	0.74	0.09	0.12	0.90	15

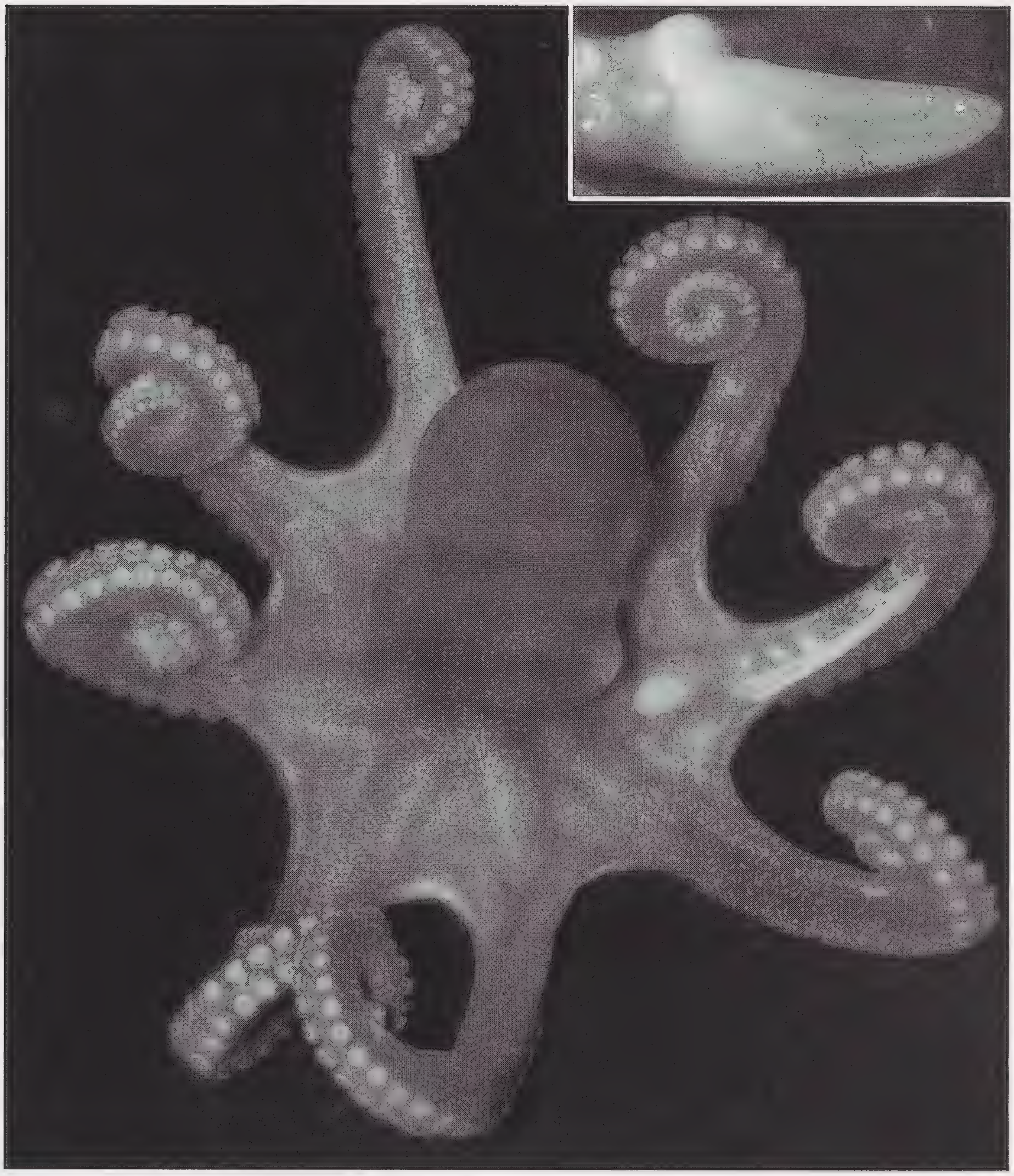

FIG. 4. Shipboard photograph of a live animal of ***Benthoctopus rigbyae*** n. sp. (by E. Jorgensen, 2007). Note nearly uniform color, lack of papillae and lack of skin components. Inset: Close-up of the hectocotylized arm tip of a mature male.

comprising 70–90% of total length, generally 3–4 times ML. Order of arm length variable but non-hectocotylized arms typically subequal in length, with 113–165 suckers. Variability in sucker counts partly a result of difficulty in counting very many pairs of miniscule suckers on arm tips. Hectocotylized right ventrolateral arm (Fig. 6a) length approximately 75% of opposite arm length. Sucker number on hectocotylized arm 61–78. Suckers small, maximum diameter 1–3% of arm length or approximately 7.5% ML. Sucker series widely separated, with zigzag pattern of transverse ridges and grooves between adjacent suckers (Fig. 6a). Distinctly enlarged suckers absent. Ligula with indistinct median ridge and numerous faint transverse creases that sometimes are barely visible. Ligula length approximately 10% (6–16%) of hectocotylized arm length or 26% (18–30%) of ML. Calamus sharply pointed, length approximately half (0.46–0.50%) of ligula length. Funnel length approximatrely 1/3–1/2 ML; length of free funnel approximately 20–40% ML.

Internal Anatomy: Funnel organ W-shaped, with moderately thick limbs. Chromatophores present in lining of buccal cavity. Beaks as in Figure 6e–g. Radula (Fig. 6j): rachidian multicuspid, 2 pairs laterals small and triangular, 2nd larger than 1st, 1 pair marginal teeth curved

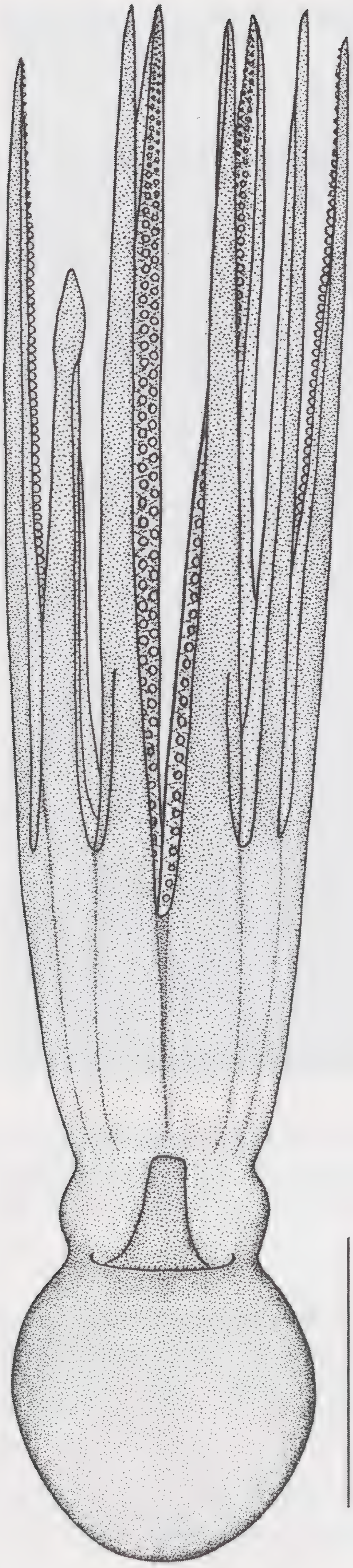

FIG. 5. ***Benthoctopus rigbyae*** n. sp. external anatomy, ventral view, male, 70 mm ML. Scale bar = 50 mm.

and much larger than laterals, 1 pair marginal plates. Dorsal visceral membrane densely covered with chromatophores. Digestive gland (Fig. 6h, i) almost spherical but slightly flattened dorso-ventrally, with shallow depression for esophagus, stomach and caecum, cream to deep maroon in color. Esophagus straight, slightly expanded in crop region, no crop diverticulum. One octopod when dissected had ventral area of crop region swollen and spherical with fluid. Anterior salivary glands large, dorsolateral on buccal mass. Posterior salivary glands moderately large, each similar in size to stomach. Stomach and caecum about equal in size. Intestine straight. Male reproductive tract as illustrated in Figure 6c, d. Accessory spermatophore gland longer than Needham's sac. An 80 mm ML male had 28 mature spermatophores in Needham's sac; one of 63 mm ML had 24, with lengths ranging from 55 to 102 mm (mean 94 mm). Female reproductive tract as illustrated in Figure 6b. Oviducts narrow, exit from antero-ventral membrane of ovary separately but contiguously, then bend laterally and funnel into round oviducal glands. In mature females, distal oviducts longer than proximal, white and thick, diameter only slightly less than that of oviducal gland. Mature ovarian eggs elongate, 13–24 mm long (mean 17 mm) x 3–8 mm wide (mean 6 mm). An 81 mm. ML female had 88 mature and 4 immature (3 x 8 to 3 x 10 mm) eggs in ovary.

Size at Maturity: Maturity of males increases gradually with increasing size (Fig. 7). Females, on the other hand, exhibit a wide range of maturity stages over a fairly narrow size range (Fig. 8). This may indicate that although most females appear to attain larger sizes than males, females mature rapidly once a threshold size has been reached.

Stomach Contents: Of 15 stomachs opened (n = 9 females, 6 males), eight were empty (n = 6 females, 2 males) and seven were partly filled but not distended (n = 3 females, 4 males). Two stomachs contained only amphipod remains; one contained amphipod and fish remains in approximately equal volumes; one contained half amphipod remains together with the remains of serolid isopods, polychaetes, bryozoans and sponge spicules. One stomach contained solely the remains of ophuroids. Two stomachs contained unidentifiable crustacean remains.

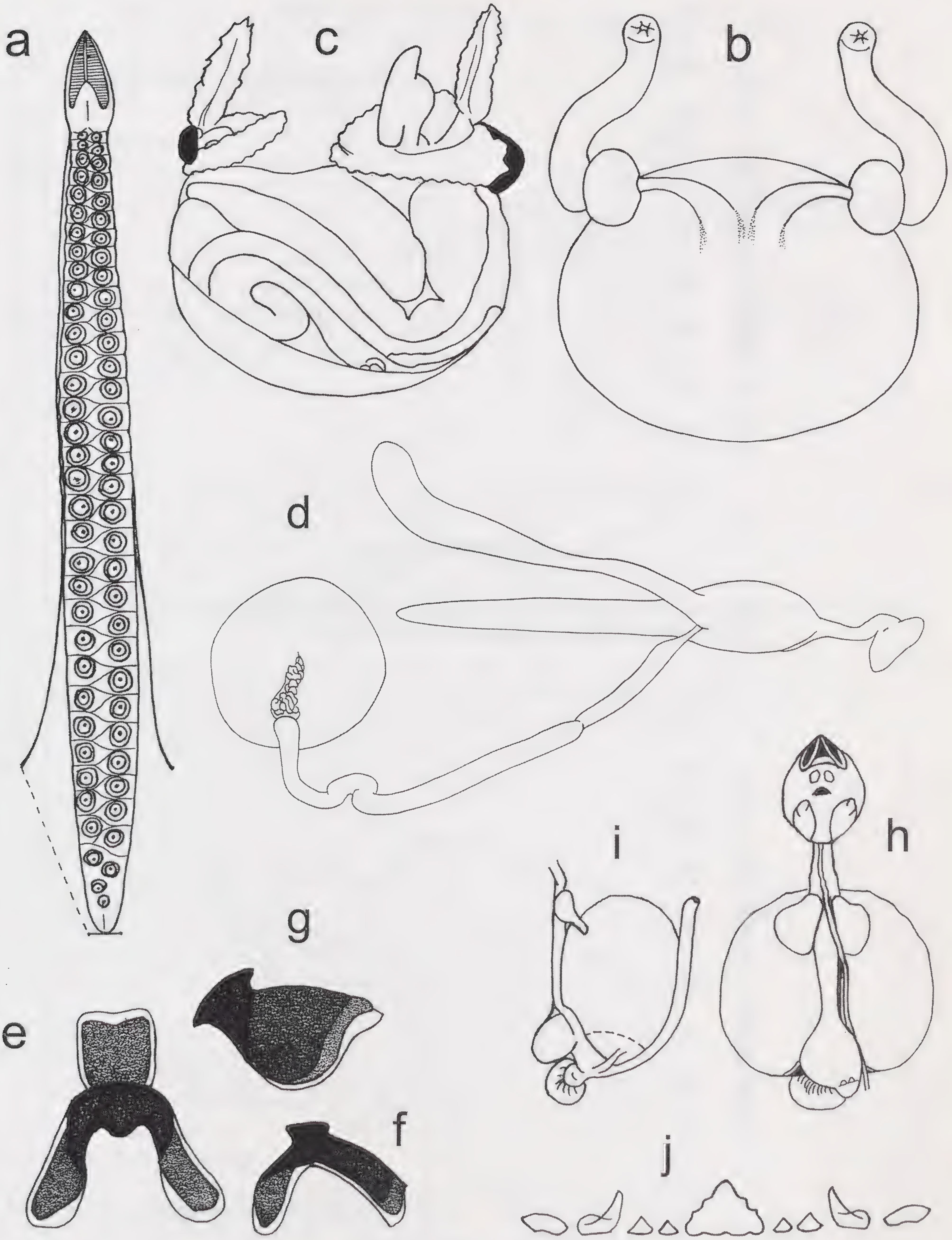

FIG. 6. ***Benthoctopus rigbyae*** n. sp. Internal anatomy (a) hectocotylized arm, (b) female reproductive system, (c) male reproductive system as seen in the viscera, (d) male reproductive system dissected to show structure, (e) lower beak, ventral view, (f) lower beak, lateral view, (g) upper beak, lateral view, (h) digestive system, dorsal view, (i) digestive system, lateral view, (j) one row of radular teeth.

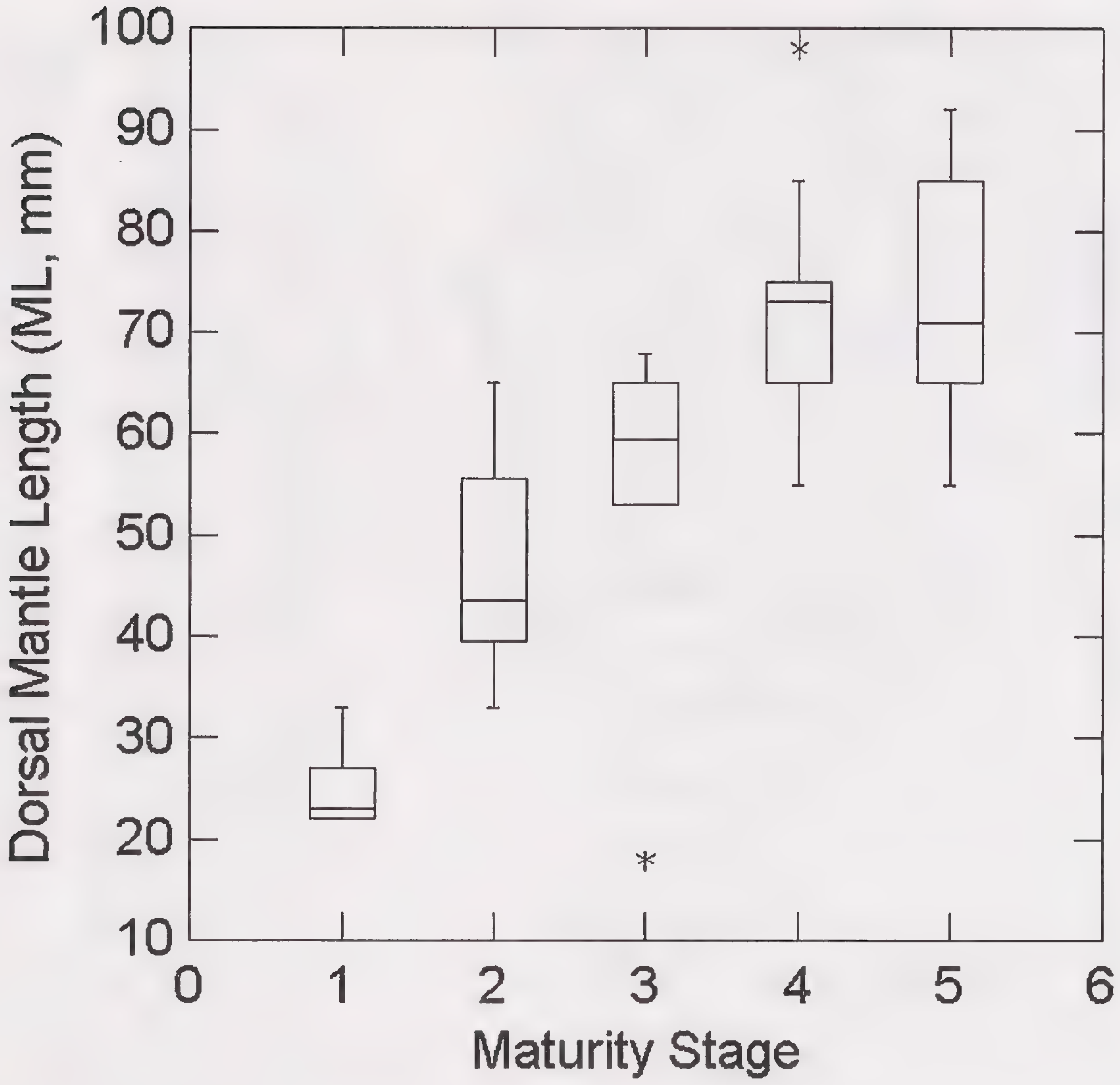

FIG. 7. Relationship between maturity stage and dorsal mantle length for male ***Benthoctopus rigbyae*** n. sp.

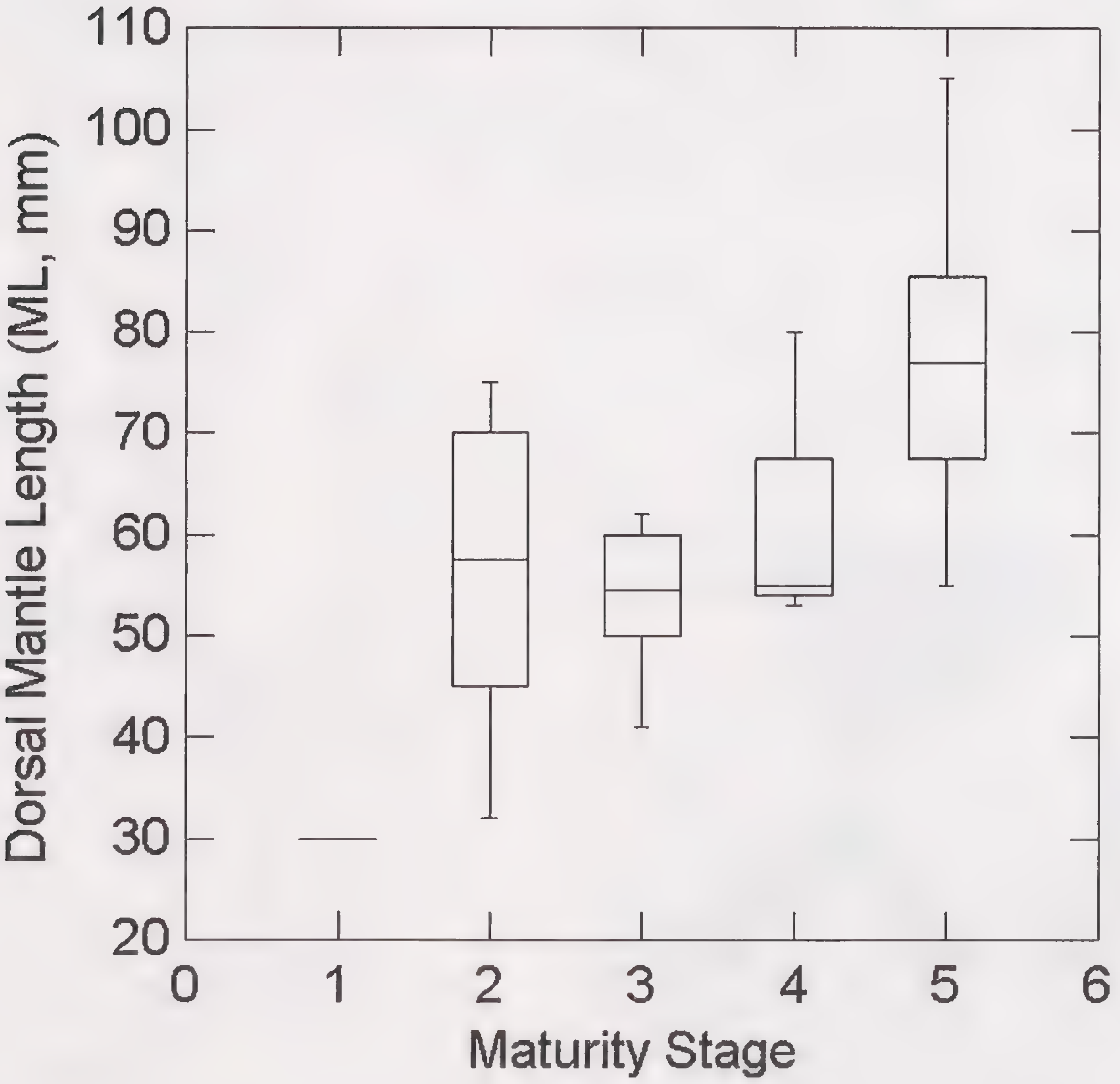

FIG. 8. Relationship between maturity stage and dorsal mantle length for female ***Benthoctopus rigbyae*** n. sp.

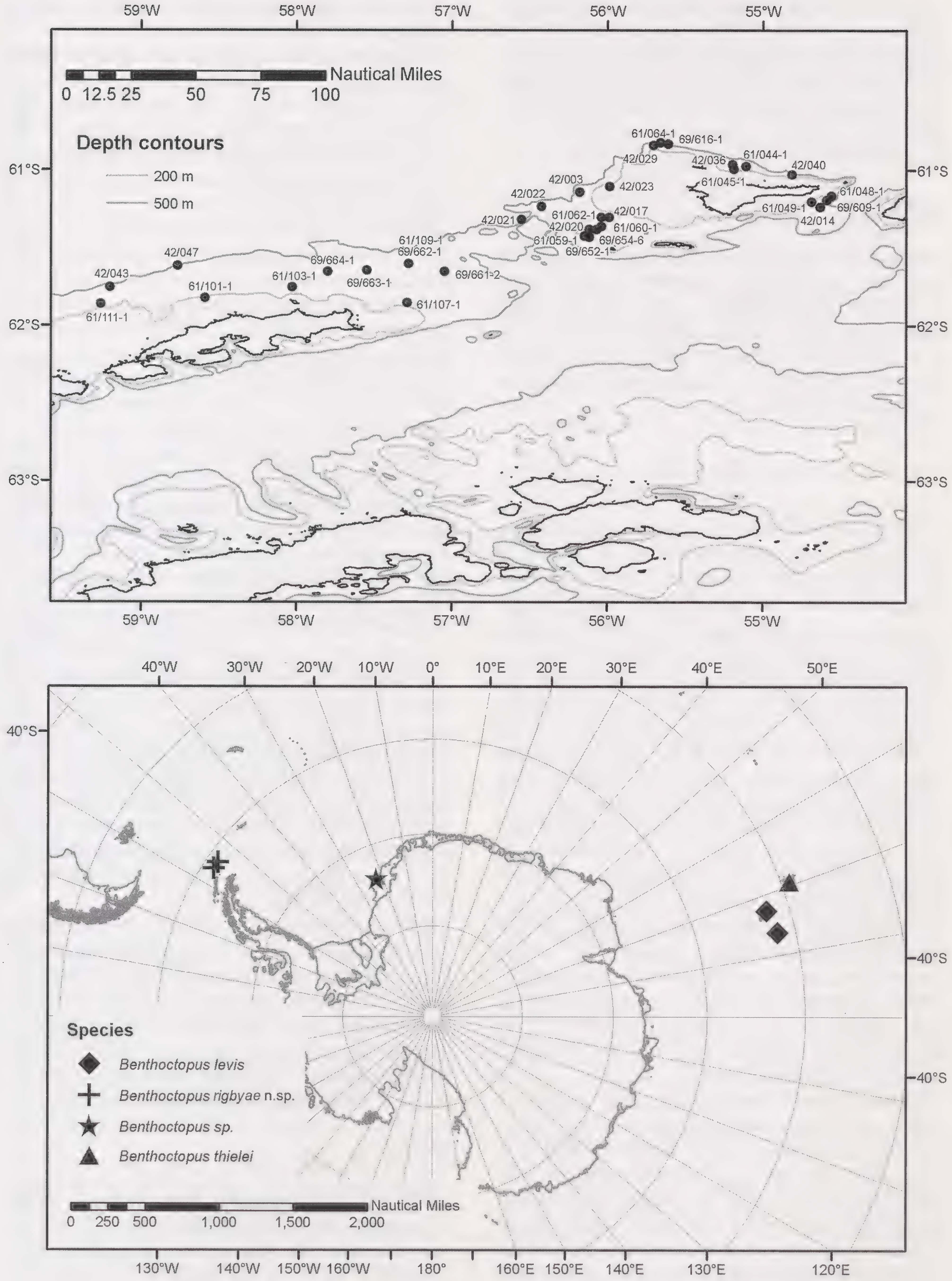

FIG. 9. Distribution of ***Benthoctopus rigbyae*** n. sp. catches in the vicinity of the South Shetland Islands (top); Distribution of *Benthoctopus* spp. around Antarctica (bottom).

Length/Weight Relationship: The relationship between total length and weight, including all maturity stages, was similar between the sexes. For females the relationship could be described by the power function $y = 0.0492x^{1.9845}$, $r^2 = 0.8984$. The function descriptive of the males is $y = 0.0023x^{2.6887}$, $r^2 = 0.9298$

Distribution

These octopods were common, though not abundant, along the outer shelf and upper slope of entire South Shetland Island chain, depth range 250–600 m (Fig. 9). They comprised < 2% of the octopod fauna sampled. The maximum number collected in a 30-min tow with the bottom trawl was 18.

DISCUSSION

Octopods with biserial suckers but lacking an ink sac currently are considered to belong to either *Bathypolypus* or *Benthoctopus.* The former is fairly well defined (Muus, 2002), based primarily on morphology of the hectocotylus, which has a very large ligula characterized by prominent transverse laminae. Although the males described here have fairly large ligulae with very faint transverse creases, their long arms, small suckers, and smooth skin make them more similar to *Benthoctopus* than *Bathypolypus. Benthoctopus,* however, has tended to be a "catch-all" taxon including any such octopod that does not clearly belong to *Bathypolypus.* The genus as currently defined probably is not holophyletic. Species of the genus *Benthoctopus* described from the area of the Antarctic Polar Frontal Zone (APFZ) or further south include just *B. theilei* Robson, 1932, and *B. levis* (Hoyle 1885). The type localities of *B. theilei* and *B. levis* lie on the APFZ off the Kerguelen Islands and south of the APFZ off Heard Island respectively.

Based on published descriptions, the specimens described here are most similar to *B. levis.* However, they differ in relative arm length, web depth, lengths of the hectocotylized arm and ligula. One of us (L.A.) examined the holotype of *B. levis* (BMNH 1889.4.24.43) and found that its funnel organ is VV-shaped rather than W-shaped. A photograph of *B. levis* from near the type locality published by Norman (2000) shows an octopod similar to ours, but with shorter arms and a deeper web. ***Benthoctopus rigbyae*** is easily distinguished from *B. thielei* as the latter has a VV funnel organ (illustrated by Robson, 1932: 234).

Although similar to *B. levis,* the specimens we describe differ sufficiently in morphology for us to believe they represent a separate species. This conclusion is supported by the molecular data. ***Benthoctopus rigbyae*** n. sp. is the sister taxon to *Benthoctopus* sp. from the eastern Weddell Sea, whilst *B. levis* is the sister taxon to *B. thielei*. The Weddell Sea specimen differs from ***B. rigbyae*** by 18 base pairs in COI the DNA bar-coding gene. We must therefore conclude that the Weddell Sea specimen also represents a different, currently undescribed species. Measurements taken shortly after capture support this. However, this specimen was destroyed due to mechanical failure of a freezer prior to fixation and is no longer extant.

To discover two species in the western Southern Ocean is not surprising given that these *Benthoctopus* species appear to inhabit shallower waters than they do elsewhere. ***Benthoctopus rigbyae*** is known from 200–600 m and the Weddell Sea specimen was captured at 850 m. Between the South Shetland Islands and the eastern Weddell Sea, water depths exceed the maximum known depth for the species. Given that mature ovarian eggs of ***B. rigbyae*** have been recorded at 24 mm length and undoubtedly hatch into benthic crawl-away young, opportunities for gene flow between the Peninsula region and the eastern Weddell Sea would be extremely limited. Thus, there is limited possibility of a continuous population around the Antarctic continent because of the Filchner-Ronne Ice Shelf which extends out over water depths greater than 1,000 m.

It is perhaps more surprising to find clearly differentiated species between the Kerguelen Islands and Heard Island. The geographic distance between these islands is small and both are situated on the Kerguelen plateau meaning that there is no deep water trench between them. It is possible that the APFZ in this region is influential in delimiting two distinct microhabitats.

Etymology

Named in memory of Robin Rigby, a bright, young cephalopod biologist.

ACKNOWLEDGMENTS

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LIFE HISTORIES AND DYNAMICS OF STREAM AND LACUSTRINE POPULATIONS OF *MUSCULIUM ARGENTINUM* (D'ORBIGNY, 1842) (BIVALVIA: SPHAERIIDAE) FROM SOUTHERN CHILE

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ABSTRACT

Life histories and population dynamics of two populations of *Musculium argentinum*, one inhabiting a fluvial environment and the other a lacustrine one, are compared. *Musculium argentinum* is oviparous, iteroparous, and sequential incubator, reproducing throughout the year. The life cycle pattern of the fluvial population, unlike the lacustrine one, is characterized by seasonal reproductive variations, more marked in spring and summer, higher fertility and population reproductive productivity (potential recruitment), higher number of marsupial sacs per hemibranch, and smaller size of first reproduction. The content of organic matter and water flow would be the main factors determining the higher population density observed in the fluvial population.

Key words: *Musculium*, Sphaeriidae, population dynamics, freshwater bivalves, Chile.

INTRODUCTION

Sphaeriid clams have a cosmopolitan distribution in a variety of temporary and permanent fluvial and lacustrine freshwater environments (Way & Wissing, 1982). This ubiquity is due to life-history traits involving strategies that allow its permanence, in time and space, in highly unpredictable environments, including the simultaneous hermaphrodite condition, with the possibility of self-fertilization, which allows them to colonize and maintain populations in temporary freshwater bodies (Hornbach et al., 1980, 1982, 1990; Morton, 1985; Hornbach & Childers, 1986; Way, 1988).

Both iteroparity and semelparity are known, with lifespans usually between five and 24 months. In addition, intra- and interspecific variations in brood size are known, yet rarely has an explanation for these variations in life-history and reproductive potential been advanced (Mackie et al., 1976a, b; Mackie, 1978, 1979).

In Chile, sphaeriids are represented by three genera and 11 species: *Psidium* with seven species, *Sphaerium* with two species, and *Musculium* with two species (Parada & Peredo, 2002). The presence of *Musculium argentinum* (d'Orbigny, 1842) in Chile was reported for the first time by Sobarzo et al. (2002) in the Cautín River basin; Parada et al. (manuscript under review) report that *M. argentinum* is found discontinuously in Chile between 38–41°S and 71–72°W, inhabiting streams and lacustrine environments. Recently, Peredo et al. (2007) reported on population dynamics and reproduction of *Musculium argentinum* from a lotic environment. To date, the population dynamics and reproductive traits of lacustrine populations of *M. argentinum* have not been described. In the present paper, a comparative study of life histories and population dynamics of stream and lacustrine populations of *M. argentinum* from humid environments in southern Chile is reported.

MATERIALS AND METHODS

Study Sites

The present study was carried out in two sites with different environmental conditions:

(1) Lautaro stream, (L) corresponds to the evacuation system of Lautaro Fish Farm located in the central valley of southern central Chile (38°32'S, 72°27'W), at 190 m altitude; it runs to the interior of a recreation park with abundant tree vegetation, represented by *Pinus radiata* (D. Don); *Acacia melanoxylon* (R. Br.),

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Eucalyptus globulus (Labill), *Rubus ulmifolius* (Schott), and riverside vegetation, with predominance of *Leontodon taraxacoides* (Vill) Merat, *Plantago lanceolata* (L.), *Taraxacum officinalis* (Weber), and *Blechnum hastatum* (Kaulf). With about 2 km longitude and a mean width of 2 m, the stream flows into the Cautín River.

(2) Lake Villarrica (39°16'27"S, 71°58'54"W) in the Andes foothills, at 250 m altitude. This lake, of glacial origin, has been characterized as oligotrophic, temperate, monomictic, with winter circulation and summer stagnation (Campos et al., 1983). Mean winter temperature is 9.5°C, with minor variations between surface and bottom. The maximum temperatures recorded in spring summer reached 22°C in the epilimnion and 9.5°C in the hypolimnion. The chemical and physical water parameters as well as the phyto- and zooplankton assemblages show marked seasonal variation. *Musculium argentinum* inhabits the coastal area of La Poza Bay (LPB), at the northeast corner of the lake, an area with vegetation of *Juncus procerus* (E. Meyer) and *Salix viminalis* (L.) and with high anthropic influence during the summer.

Biological Parameters

From October 2006 to October 2007, the two studied populations of *M. argentinum* were sampled monthly. The biological material collected (70 specimens per month) was transferred to the laboratory to measure the shell length (SL) of each specimen using a digital caliper. Twenty specimens of known SL were selected at random to determine the presence and number of brooding sacs within inner hemibranchs, number of embryos per sac, and number of extramarsupial embryos. In October 2006, at each location, population density was calculated with ten 400 cm^2 random samples of substratum. Each sample was sifted through 1 mm mesh sieve and specimens in each sample counted. Results are expressed as ind/m^2. Population size structure was determined by analyzing monthly size frequency distributions; size (= age) of first brood was determined at individual and population level. Due to climatic reasons, it was not possible to carry out the sampling in July at LPB. Spearman coefficient (r_S) was used to observe correlation between shell length (SL) and number of brooding sacs present in gravid adults.

Physical and Chemical Parameters of Water and Substratum

In October 2006, at each location, water samples were taken to determine total suspended solids, dissolved oxygen, total alkalinity, pH, total ammonium, nitrate, chloride, calcium, phosphorus, and conductivity. Water samples were processed according to the methodology established by APHA et al. (1992). The flow and depth of the channel and the monthly temperature of water were determined in situ.

To determine the granulometric characteristics and percentage of organic matter in the substrate, in October 2006, at each location, four additional samples of substratum (volume = 800 cc) were

TABLE 1: Population and reproductive parameters of *M. argentinum* stream and lake population and water temperature during the study period.

Parameters	Lautaro Stream population	La Poza Bay Lake population
Maximun shell length (mm)	8.0	7.8
Density (ind/m^2)	325.0	187.5
Maximun individual Fertility	26	18
Mean annual Productivity	96 ± 39	78 ± 25
Maximun number of brooding sac per gill	26	18
Maximun number of embryos per sac	3	2
Mean annual embryos	77.2 ± 15.7	64.9 ± 19.4
Mean annual extramarsupial embryos	19.0 ± 15.3	13.1 ± 10.5
Individual size first reproduction (mm)	2.3	2.8
Mean annual water temperature (°C)	10.6	15.1

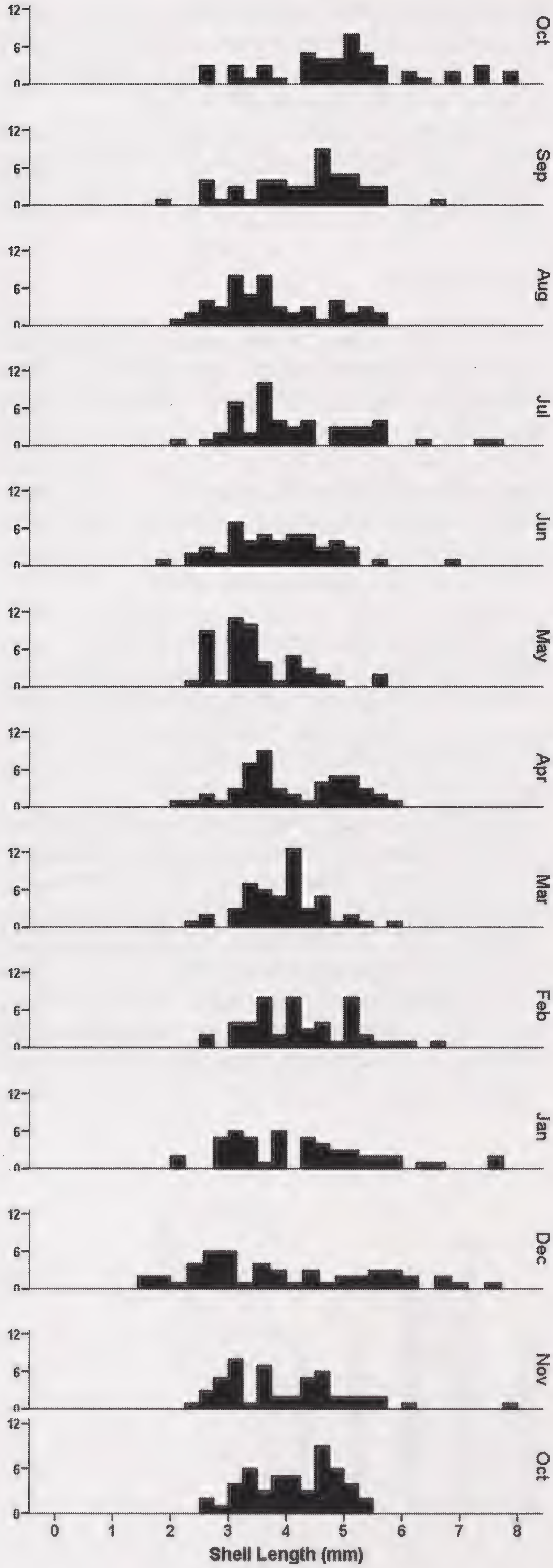

FIG. 1. Monthly size structure of *Musculium argentinum* population at Lautaro stream.

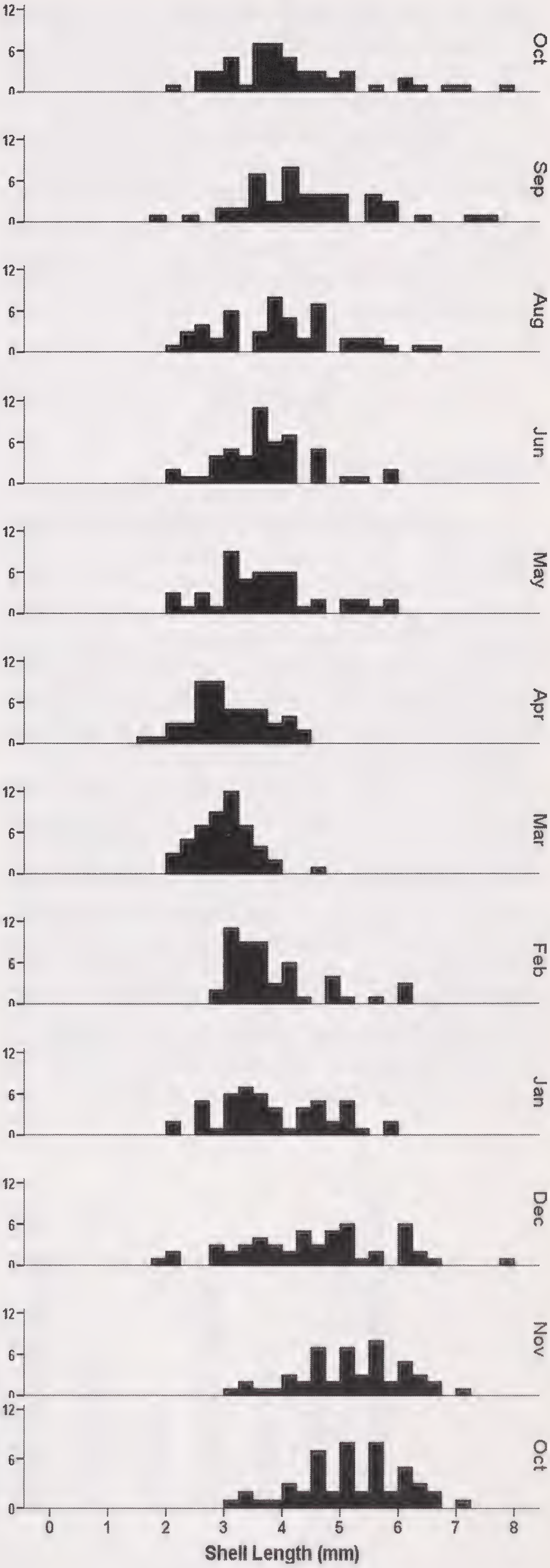

FIG. 2. Monthly size structure of *Musculium argentinum* population at La Poza Bay.

taken. In the laboratory, samples were dried in a Memert oven for 48 h at 110°C. Afterwards each sample was weighed before and after being maintained in a muffle at 500°C for five h to determine content of organic matter. Each substratum sample was sieved through a series of 0.787 mm, 0.278 mm, and 0.083 mm sieves to separate fractions of sand, mud and clay. Differences in granulometry of sampling station substrata were determined according the Textural Triangle (USDA, 1975).

RESULTS

Population Dynamics

From a total of 840 specimens from the lake population, the smallest individual (SL = 1.8 mm) was recorded in December and the largest specimen (SL = 7.8 mm) was recorded in October 2007. In the stream population from a total of 840 specimens collected, the smallest individual (SL = 1.8 mm) was recorded in September and the largest (SL = 8.0 mm) was recorded in October 2006 (Table 1).

Figures 1 and 2 show the monthly size structures of stream and lake populations. Both populations showed similar monthly size-frequency distributions. In the stream populations, the widest size-classes interval appeared in October 2007, whereas in the lake population they appeared in December. Juveniles (specimens with SL of 1.0–1.9 mm) were present throughout the year, being more abundant in the lake population. Figure 2 shows that in the lake population December 2006 and April and September 2007 are the months with the highest number of juveniles, whereas for the stream population the highest number of juveniles were in June and September 2007 (Fig.1).

Population density of *M. argentinum* in the stream population was 325 ind/m^2. On the other hand, the lake population density was 187.5 ind/m^2 (Table 1).

Reproduction

During the spring and summer months, the monthly variation in the number of gravid individuals showed a similar pattern in both populations, that is, a progressive increase in the number of gravid individuals, reaching a peak in January, to decrease towards March, when the lowest values were found. During fall and winter seasons, the reproductive behavior of each population was different: the lake population tends to reach the mean percentage in May, June, August and September over 50% of gravid individuals. Instead, during the fall months, the stream population showed a percentage of gravid individuals below 40%, with an increase in July and a decrease in September (Fig. 3).

Adults with brooding sacs were present throughout the year in both populations. However, extramarsupial embryos were not recorded in November and August for stream population samples, and in January, February and July in the lake population samples. The stream population showed gravid individuals with up to three sacs per demibranch during December and October. In the lake population, the maximum number of brood sacs per hemibranch was

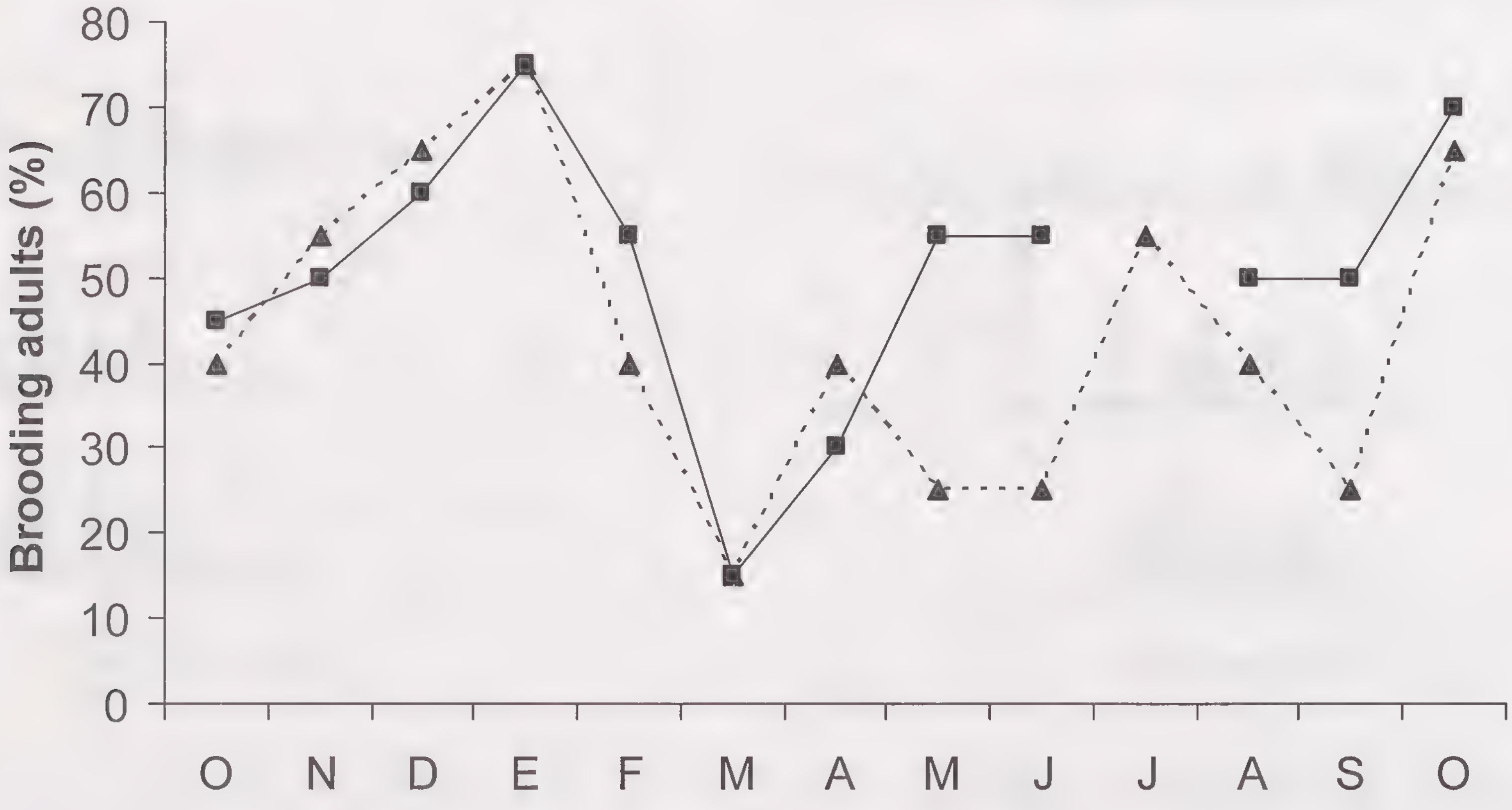

FIG. 3. Monthly variation of the percentage of gravid specimens at Lautaro (triangles) and La Poza Bay (squares) populations.

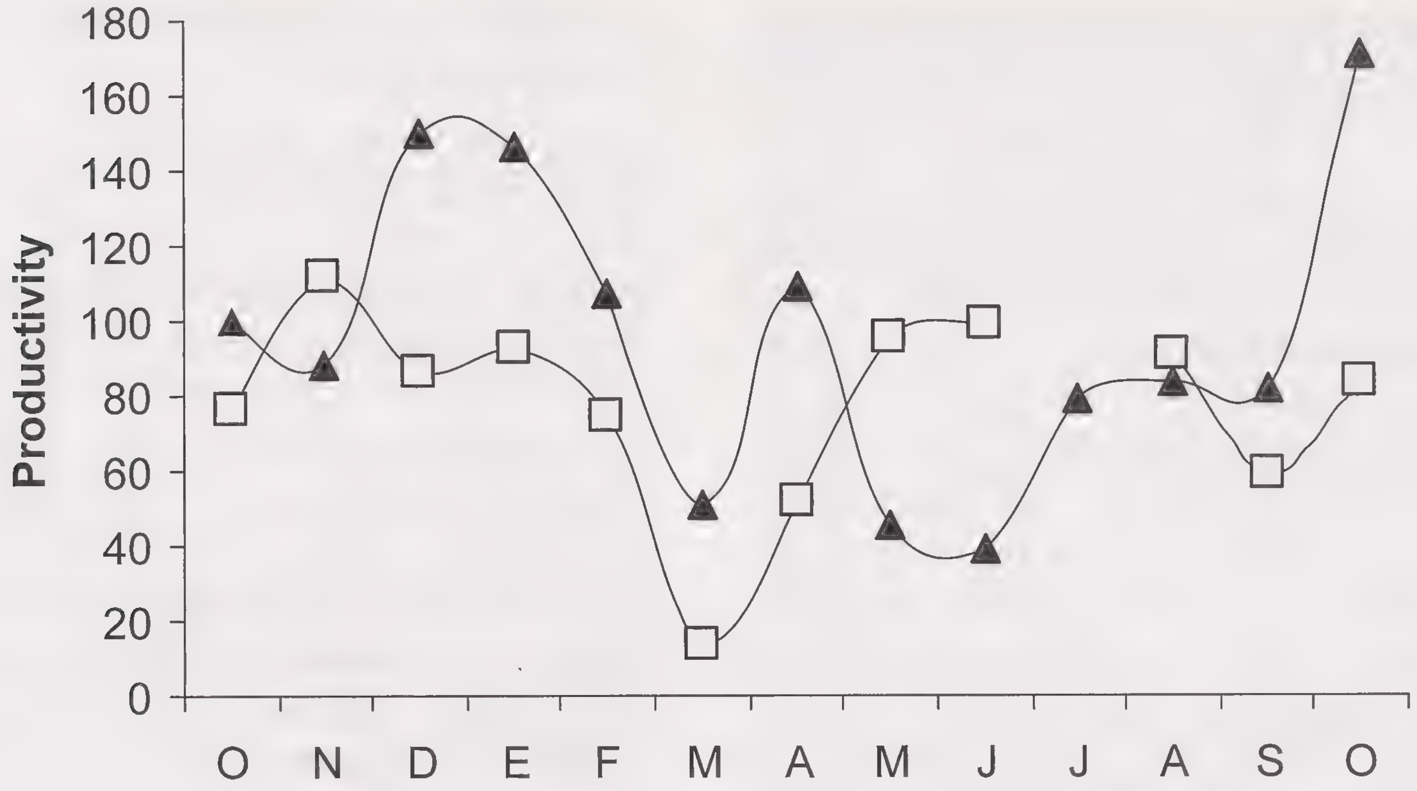

FIG. 4. Monthly potential productivity (number of within-sac embryos plus extramarsupial embryos) in Lautaro (stream) (triangles) and La Poza Bay (lake) (squares) populations.

two (Table 1). In both populations, there is a low positive, significant (P = 0.001) correlation between SL and number of brood sacs in gravid adults (L $r_S = 0.48$; LPB $r_S = 0.31$).

The mean values of annual productivity (or potential recruitment) measured as the number of embryos contained in brood sacs and extramarsupial embryos within the suprabranchial chamber produced by adults was: 96.2 ± 39 embryos in the stream population and 78 ± 25 for the lake population (Table 1). Monthly variations of this parameter for the populations under study are shown in Figure 4. Although both populations showed a similar tendency throughout the year, values of potential recruitment for the stream population were consistently higher that those corresponding to the lake population, with the exception of May and June, when the productivity in stream population was lower. At the individual level, monthly variations of maximum individual fertility (measured as the number of intra- and extramarsupial embryos produced by a single individual) in each population are shown in Figure 5. The stream population showed the highest values, 26 embryos, in April and October, corresponding to maternal individuals of 4.5 mm and 6.2 mm SL respectively. In the lake population, the highest value of fertility was 18 embryos in January for a maternal individual of 4.7 mm SL (Table 1).

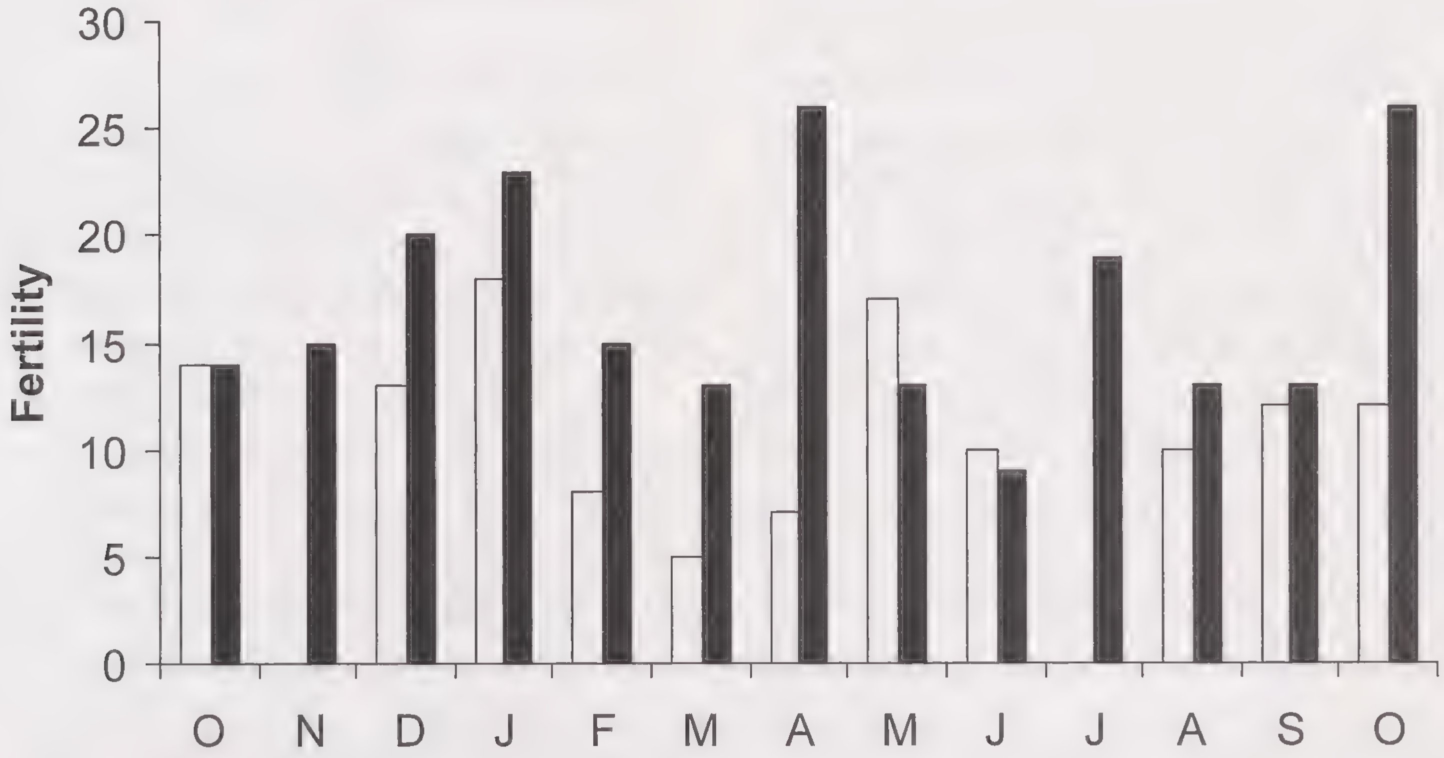

FIG. 5. Monthly variation of highest individual fertility (specimen with the highest number of within-sac embryos and extramarsupial embryos) in Lautaro (stream) (black) and La Poza Bay (lake) (white) populations.

TABLE 2: Water physical-chemical parameters at Lautaro and La Poza Bay sampling stations during the study period.

Parameters	Lautaro	La Poza
Total suspended solids (mg/l)	1.5	1.6
Conductivity (µS/cm)	62.1	57.9
Dissolved oxygen (mg/l)	11.54	10.13
pH	7.71	7.79
Alkalinity (mg/l)	26.50	28.35
Calcium (mg/l)	5.73	0
Total ammonium (mg/l)	0.024	0.007
Nitrite (mg/l)	0.003	0.050
Nitrate (mg/l)	0.06	0.06
Chloride (mg/l)	0.7	1.3
Phosphorus (mg/l)	0.03	0.01
Sulphate (mg/l)	0	6
Temperature (ºC)	10	13
Deep (cm)	15.7	30.0
Water flow (m/s)	55	0

Table 2 shows the minimum size of brooding individuals of *M. argentinum* obtained monthly for each population. The results show that the smallest gravid individual in the stream population was 2.3 mm SL and in the lake population 2.8 mm SL, collected in May (Table 1). The size of first brooding at the population level varied seasonally. The smallest size class for which 50% of individuals were gravid corresponded in both populations to the size class of 3.0–3.9 mm recorded in August and July for the stream population and in February and May for the lake population.

Habitat Characteristics

The physical and chemical characteristics of the substratum at each study site showed differences in the organic matter content and granulometry. At the stream station, the substratum had a considerably higher percentage of organic matter (11%) than the lake station with only 1.5%. Both stations also showed differences in granulometry. At the stream, the substratum was predominantly mud and clay, with 0.91% of sand (54% of mud and 45.1 of clay); at the lake, a clay fraction as dominant (81%), with 7.3% of sand and 12.2% of mud.

Table 2 shows the other physical and chemical parameters at lake and stream. The water quality of both habitats is similar with differences in the values corresponding to chloride and nitrite, particularly at the lake, where differences are more marked. Ammonia and phosphorous levels are 3–4 times higher in the stream than the lake. Calcium was only present at the stream. Differences between the habitats are great, especially due to the depth fluctuations in the coastal areas of Lake Villarrica, reaching the minimum, 30 cm, in summer and a maximum of 2.5 m in winter; the lake is also characterized by the lack of water circulation, in contrast with the stream, where the water flow is 0.5 m/s, without significant variations throughout the year. Monthly variations of water temperature in the habitats showed the same tendency throughout the year, however, at the stream, temperatures were consistently lower (Fig. 6).

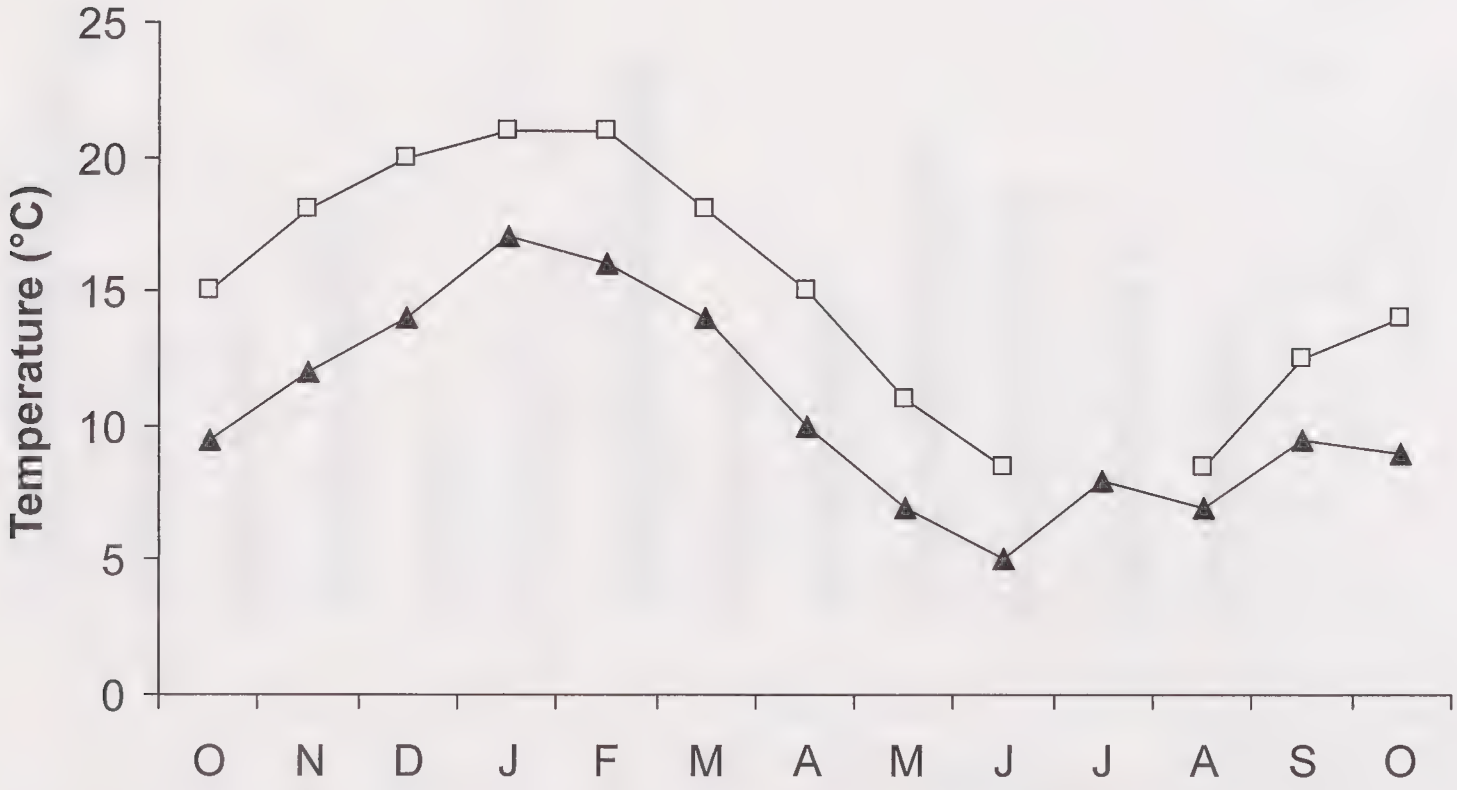

FIG. 6. Monthly water temperature at Lautaro (triangles) and La Poza (squares) during the study period.

DISCUSSION

All individual experience environmental variations in abiotic or biotic conditions. Such environmental variation has a strong impact on the fitness of individuals and thus strongly influences the evolution of life-histories strategies.

The present study of the life histories of stream and lacustrine populations of *M. argentinum* showed a similar pattern in the annual variation of the size-frequency distributions; brooding was observed in a broad interval of size classes (2.3 to 8.0 mm SL) throughout the year. *Musculium argentinum* is an ovoviviparous, iteroparous, and sequential brooder. These results correspond to those reported by Peredo et al. (2007) for *M. argentinum* in other localities of southern Chile. Iteroparity in *M argentinum* populations could be indicative of a strategy to favor reproductive success, as indicated by numerous models (Wilbur & Rudolf, 2006) Nevertheless, some substantial differences between stream and lacustrine populations were observed (Table 1). Although in both populations incubation and recruitment were recorded throughout the year, the stream population showed a more defined seasonal variation in the annual reproduction, with higher values in the frequency of gravid individuals in spring and summer (October to February, with a maximum in January) and a noticeable decrease in fall and winter (Fig. 3). In contrast, a more uniform brooding behavior throughout the year was observed in the lake population, for which only one noticeable decrease in the number of gravid specimens was observed in March, with a relative stable number of gravid adults during the rest of the year.

Furthermore, specimens from the stream population showed within each size class a higher number of embryos contained in brood sacs and extramarsupial embryos, which explains the higher values of individual fertility and productivity or potential recruitment observed in the stream population (Figs. 4, 5).

Specimens of from the stream population produced up to three brood sacs per hemibranch; instead, the maximum number of sacs per demibranch in the lake population was two (Table 1). In both populations, there was low positive, significant (P = 0.001) correlation between the number of sacs produced and shell length of maternal specimens, a fact reported by Peredo et al. (2007). Beekly & Hornbach (2004) reported that in *Sphaerium striatinum* (Lamarck, 1818) the brood size is limited by two general constraints, the marsupial sac and the retention of extramarsupial offspring. In our study, specimens from the stream population, in which the maximum number of brood sacs/demibranch was higher, showed higher values in the convexity index or shell roundness (sensu Ituarte, 1996) (65.4 for the stream and 59.1 for the lake) (Parada et al., under revision). This means that, compared with the lake population, the stream population specimens have more globose shells; this morphometric characteristic is directly related to a larger mantle cavity volume, a factor that would result in a higher capacity for lodging offspring.

Another difference between stream and lake populations was found in the smallest size at which an individual is ready for brooding; 2.3 mm SL in the stream population and 2.8 mm in the lake population (Table 1). In both populations, the smallest gravid individuals were observed in May. This suggests that specimens from stream population reached mature reproductive condition earlier than those from lake population.

Mackie et al. (1976) reported that inter- and intrapopulation variations in the fertility of *Musculium securis* (Prime, 1852) could be explained by differential mortality of embryos during the incubation period. This was not the case *of M. argentinum* populations studied here, in which all dissected brood sacs observed monthly, dead embryos or remains of dead embryos were never found. There are no reports that embryo mortality in *Musculium* can be attributed to development retention to provide nutrition to other embryos, as reported in marine mollusks (Gallardo & Garrido, 1987).

The themes of density-dependent regulation, resource availability, and environmental fluctuations are integral to current demographic theory and are potentially important in any natural system (Reznick et al., 2002). The interpopulation differences in fertility recorded in *M. argentinum* could be explained rather as a response to differences in habitat characteristics. Differences in food supply represented by the significantly higher quantity of organic matter available for the stream population (11%) versus 1.5% in the lake population in the sediment used as food could be a factor that could influence fertility and productivity. The higher fertility recorded in the stream population could be advantageous to compensate for the loss of juveniles swept away by the water current (Parada et al., under review). The importance of the organic matter in the sediment has been documented for *M. lacustre* (Müller, 1774) (Morton, 1985;

Mouthon, 2004) for *M. securis* by Mackie et al. (1976a) and for *Sphaerium striatinum* by Raikow & Hamilton (2001).

Reproductive traits shown by both populations (gravid adults throughout the year) do not allow determination of the reproductive effort per year. According to Mackie (1979), *M. argentinum* has a continuous food supply and energy allocated mainly to reproduction.

Many authors, empirically as well as theoretically, have tried to correlate life-history traits of different species – age of the first reproduction, fertility, iteroparity or semelparity, growth rate, and generational time, amongst others – with habitat characteristics (MacArthur, 1960; Cody, 1966; Pianka, 1970; Southwood et al., 1974; Wilbur et al., 1974; Stearns, 1976; Parada et al., 1990; Parada & Peredo, 1994).

Population densities of *M. argentinum* at the stream and the lake showed marked differences: 325 ind/m^2 and 187.5 ind/m^2, respectively. In this context, the environmental parameters represent relevant factors in the dynamics, reproductive biology, and permanence of populations in time. It is just as a conjunction of parameters are observed in common where the populations of *M. argentinum* are located; the water registers maximum averages of 23°C and minimum of 8°C, low values of total dissolved solids, low conductivity, waters highly oxygenated and, neuter pH, with low values of ammonium, nitrite, nitrate chloride, sulphate, inorganic phosphorus, and calcium, and differences in organic matter content in the substratum (Table 3). These values are much lower than those recorded in Saone River in France for *Musculium lacustre* (Mouthon, 2004). The studied populations of *M. argentinum* occur in water bodies with clay (lake population) or clay- muddy bottoms (stream population), with organic matter contents ranging from 11.03 to 1.52% for L and LPB respectively. From the above mentioned environmental parameters, content of organic matter and water flow would be the main factors determining the higher population density observed in the stream population.

The low or absent calcium in Lautaro stream and La Poza Bay water is remarkable. Burky et al. (1979) suggested that sphaeriid clams have a common strategy for shell secretion that is presumably under genetic control. Energetically, it would seem most economical for clams to build the most calcareous shell in the hardest water or the most proteinaceous shells under the most eutrophic conditions. However, in terms of water hardness, this hypothesis must be rejected since interspecifically there is no relationship between shell calcium and water hardness, and there is an inverse relationship for *Sphaerium striatinum.*

Although no water characteristic can explain the relationship between the calcareous and organic components of shell, trophic conditions give the best explanation at the generic level, because *Musculium* is at the opposite end of the eutrophic scale from all other sphaeriids that have been examined. The inverse relationship between the amount of shell calcification and water hardness for *S. striatitum* is indicative of selection for other patterns of shell secretion at the species level. At the same time, data on populations of *Musculium*, *Sphaerium* and *Pisidium* suggest selection for shell of a base level of adaptative need and mechanical function (inverse relation between shell organic and shell $CaCO_3$) before the splitting of the Sphaeriidae into genera.

The *Musculium argentinum* studied inhabit soft waters and with absence or very low calcium concentration. The physical-chemical parameters of the water where *M. argentinum* populations live corresponds to an oligotrophic to mesotrophic environment. However, according to the organic matter recorded in the substratum, especially that present in Lautaro stream, corresponds to a eutrophic environment. *Musculium argentinum* in contrast to *Sphaerium striatinum,* lives in softwater, with low calcium concentration level which does not affect shell formation, thus corroborating Burky et al. (1979) observation with respect to the inverse relationship between the amount of shell calcification for *S. striatinum* being indicative of selection for other patterns of shell secretion at the species level.

Selection for life in variable habitats has evoked speculation about of the deterministic r and K selection theory to life history tactics (Pianka, 1970; Hornbach et al., 1980; McLeod, 1981). Heard (1977) concluded that some sphaeriidae species are r strategists because they produce many young of low energy content and live in variable habitats. In contrast, other species of Sphaeriidae are K strategists, occupying more stable habitats and producing few offspring of high energy content. Results of the present study do not allow characterization of either population as r or K strategists because reproduction traits and environmental conditions do not fit with features of r or K strategies. Hornbach et al. (1980), Way et al. (1980), and McLeod et al. (1981) pointed out that data on *M. partumeium* (Say, 1822) seem to support a bet-hedging strategy. According to our study, *M. argentinum* would also be a bet-hedging strategist, but each population could have its own strategy, as pointed out by Burky (1983).

Musculium argentinum has a wide, discontinuous distribution in southern South America, from southern Brazil (Mansur et al., 1991, Ituarte, 1996) to Montevideo, Uruguay (Figueiras, 1965), and it is widely distributed in Argentina (Ituarte, 1996) and Chile, occupying different biotopes and climates. This species thus is an attractive study-model for the analysis of a variety of ecological and historical circumstances that influence and life histories in different environmental conditions.

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WILHELM DUNKER, HIS COLLECTION, AND PTERIID SYSTEMATICS

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ABSTRACT

Wilhelm Dunker was the foremost expert on the systematics of the bivalve family Pteriidae (referred to as genus *Avicula* in his time) in the nineteenth century. His work on the group culminated in the monograph *Die Gattung Avicula*, a part of the significant conchological iconography, *Systematisches Conchylien-Cabinet* (Küster et al., 1837–1920). Dunker's volume remains unsurpassed in the taxonomic scope and the quality of illustrations. However, despite the fact that *Die Gattung Avicula* was readily available and widely cited, few specialists consulted the actual type material from the Dunker collection, which fell into oblivion for over a century. In this work, we attempted to reconstruct the contents and the history of Dunker's collection of pteriid bivalves, the specimens of which derive from two principal repositories, the Museum für Naturkunde in Berlin and the Aquazoo-Löbbecke Museum in Düsseldorf. In addition to the annotated catalog of type and figured specimens, many of which are here recognized for the first time, this contribution gives an account of Dunker's life and science, and provides an overview of pertinent contemporary shell collections.

Key words: Mollusca, Pterioidea, pearl oysters, historical collections, taxonomy, systematics.

INTRODUCTION

The superfamily Pterioidea Gray, 1847: 199, is an ancient lineage of epifaunal marine bivalves distributed throughout the tropical and subtropical continental shelf regions. In the modern seas, pterioidean bivalves are integral components of a wide range of marine ecosystems, including threatened mangroves and coral reefs. Commercial cultivation of marine pearls rests largely on pearl oysters of a pterioidean genus *Pinctada* Röding, 1798.

Living Pterioidea includes four families: Pteriidae Gray, 1847; Isognomonidae Woodring, 1925: 57; Malleidae Lamarck, 1818: 424; and Pulvinitidae Stephenson, 1941: 151 (Newell, 1969; Boss, 1982; Butler, 1998; Tëmkin, 2006a). With the exception of Pulvinitidae, which has recently been revised (Palmer, 1984; Tëmkin, 2006b), the status of the other families requires taxonomic scrutiny. The revision is necessitated by recent phylogenetic studies that challenged an assumed monophyly of these groups (Steiner & Hammer, 2000; Giribet & Distel, 2003; Tëmkin, 2006a). Particularly daunting is species-level revision of pterioidean taxa due to a high degree of plasticity in shell shape and color, the two principal characters upon which the traditional classification of the group was based. Whereas the state of taxonomic chaos in pterioidean systematics is widely acknowledged, no attempts for improvement have been made in recent years.

The rampant proliferation of pterioid species names was largely due to the increase in number of named species of the family Pteriidae in the early to mid-nineteenth century, when the family was typically circumscribed by the generic name *Avicula*. Introduced by Bruguière in reference to the Recent Pterioidea (Bruguière, 1792: pl. 177), *Avicula* was variably used in either a more restrictive sense corresponding to the contemporary pteriid genus *Pteria* Scopoli, 1777: 397, or as a broader category, comparable to the presently recognized family Pteriidae. Pteriid species are notorious for possessing few discrete, easily identifiable shell characters. Minor differences in color (often greatly differing among members of the same population and even through the ontogeny of a single individual), shape (particularly in the degree of obliquity and the extent of the posterior auricle), size (such as seen between juvenile and adult forms), as well as geographical origin,

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were routinely used as criteria for establishing new species names.

A few nineteenth-century authors, however, were chiefly responsible for erecting large numbers of species names of *Avicula*, typically accompanied by rather meager descriptions, albeit often provided with accurate illustrations. Three works are of principal importance: a section on *Malleacea* in Lamarck's *Histoire naturelle des animaux sans vertèbres* (Lamarck, 1819: 135–152), and monographs on the genus *Avicula* by Reeve (1857) and Dunker (1872–1880). In Lamarck's system, the genus *Avicula* encompassed contemporary pteriid genera *Pteria* and *Electroma* Stoliczka, 1871: 391, and his *Meleagrina* Lamarck, 1819: 150, was equivalent to the currently recognized genus *Pinctada* (which is now considered a senior synonym of *Meleagrina*). Reeve placed all these taxa under the name *Avicula*, whereas Dunker distinguished *Meleagrina* as a subgenus of *Avicula*.

Unlike the work of Lamarck, which contained only brief descriptions, and that of Reeve, the value of which almost exclusively rests on the illustrations, the synthesis by Dunker was far more comprehensive. It contained an extensive introduction, detailed species descriptions with discussions, synonymies, additional important commentaries at the end of the volume, and superb illustrations, sometimes more than one per species. Partly based on his prior publications on the group (Dunker, 1848, 1852), Dunker's monograph *Die Gattung Avicula* (Dunker, 1872–1880) was published as a part of one of the largest and most significant conchological iconographies, *Systematisches Conchylien-Cabinet von Martini und Chemnitz* (Küster et al., 1837–1920). In this work, he revised over one hundred species of Pteriidae and described 30 new species (of total 47 pteriid species named by Dunker). The establishment of such large number of species, "rather too many for a genus, the species of which are so variable," was criticized by Martens (1874: 172) shortly after its publication.

Surprisingly, despite the importance of Dunker's contribution and a widespread use of names established by him, few, if any, later bivalve workers consulted the actual type material from the Dunker collection. Subsequent partial or regional revisions (Jameson, 1901; Hynd, 1955; Ranson, 1961; Wang, 1978; Shirai, 1994; Hwang & Okutani, 2003) referred to the material from the Cuming collection (presently at The Natural History Museum, London) upon which Reeve's (1857) monograph was based, but did not consider Dunker's specimens, most of which are still extant at the Malacozoological Collection of the Museum für Naturkunde. A number of specimens from the Scheepmaker and the Gruner collections (presently at the Aquazoo-Löbbecke Museum, Düsseldorf) that Dunker used as a basis for establishing new species also escaped the attention of subsequent workers. It is ironic that one of Dunker's names, *Pinctada martensii* (Dunker, 1880), is frequently applied to one of the most important commercial pearl oyster species in the Indo-Pacific region, whereas Dunker's type specimens have neither been discussed nor illustrated since its original publication. Consulting the original type specimen proved instrumental for unequivocal establishing the species identity of *Pinctada longisquamosa* (Dunker, 1852) and allowed for a more accurate assessment of its geographic range in a recent revision of the species (Mikkelsen et al., 2004).

The objectives of this work are (1) to highlight the significance of Wilhelm Dunker's contribution to malacology and, more specifically, to pterioidean systematics, and (2) to compile an annotated catalog of types and figured specimens of *Avicula* from his collection. This work is a significant contribution toward the on-going taxonomic revision of pterioidean taxa (Mikkelsen et al., 2004; Tëmkin, 2006a, b), as well as a part of a systematic effort to catalog type material housed at ZMB (e.g. Köhler, 2007; Dijkstra & Köhler, 2008).

BIOGRAPHY OF DUNKER

Wilhelm Bernhard Rudolph Hadrian Dunker (b. 1809 – d. 1885; Fig. 1), a professor of geology and mineralogy, and a state official in charge of mining in Marburg, Germany, amassed an extensive shell collection that eventually became an invaluable part of the mollusk holdings at the Museum für Naturkunde in Berlin. Despite the fact that Dunker was among leading malacologists of his day, little attention has been paid to both his character and the significance of his collection. The latter is particularly surprising given the large number of type specimens it contains and, hence, its relevance to the current taxonomic practice. This section provides a biographical sketch of Dunker based on prior published biographical notes and obituaries (Anonymous, 1885; Kobelt, 1885; Koenen, 1885; Crosse & Fischer, 1886; Andrée, 1955; Schmidt, 1973), and on previously unpublished documents.

Dunker was born on February 21, 1809, in Eschwege in the eastern part of Hesse, west-central Germany. He attended a gymnasium (an equivalent of a college preparatory secondary school) first in Kassel and later in Rintelen, where his father had served as an army officer since 1815. Dunker proved to be a talented artist, and at the age of 14 he was awarded a silver medal of the Kassel Art Academy for his drawings of natural history objects. As a student, he began to collect shells, insects, and minerals. Upon passing his exams, Dunker began studying mining while working in copper mines near Riechelsdorf and coal mines near Obernkirchen. His interests expanded to include geology, mineralogy, and paleontology. In 1830 Dunker entered the University of Göttingen, where he attended lectures by the anatomist Johann Friedrich Blumenbach (b. 1752 – d. 1840), and the mathematician and astronomer Carl Friedrich Gauss (b. 1777 – d. 1855), among others. While continuing working on mining around Karlshütte und Obernkirchen, Dunker received his doctoral degree from the university in Jena in the summer of 1838.

In 1839, at the age of 30, Dunker was assigned as a lecturer of mineralogy and geology at the polytechnic school in Kassel, Hesse. The following year, he married his cousin Elise Sommer. Although they did not have children, their 45-year long marriage was apparently happy. The appointment in Kassel not only provided Dunker with access to important libraries but also brought him in contact with two renowned German malacologists of the time, Rudolph Amandus Philippi (b. 1808 – d. 1904) and Ludwig Carl [Karl] Georg Louis Pfeiffer (b. 1805 – d. 1877), who rekindled Dunker's interest in mollusks.

In the period of 1846–1878 Dunker became one of the editors (together with the vertebrate paleontologist Hermann von Meyer) of the journal *Palaeontographica, Beiträge zur Naturgeschichte der Vorwelt*. After Meyer's death in 1869, Dunker alone edited 18 volumes of the journal, several supplement volumes,

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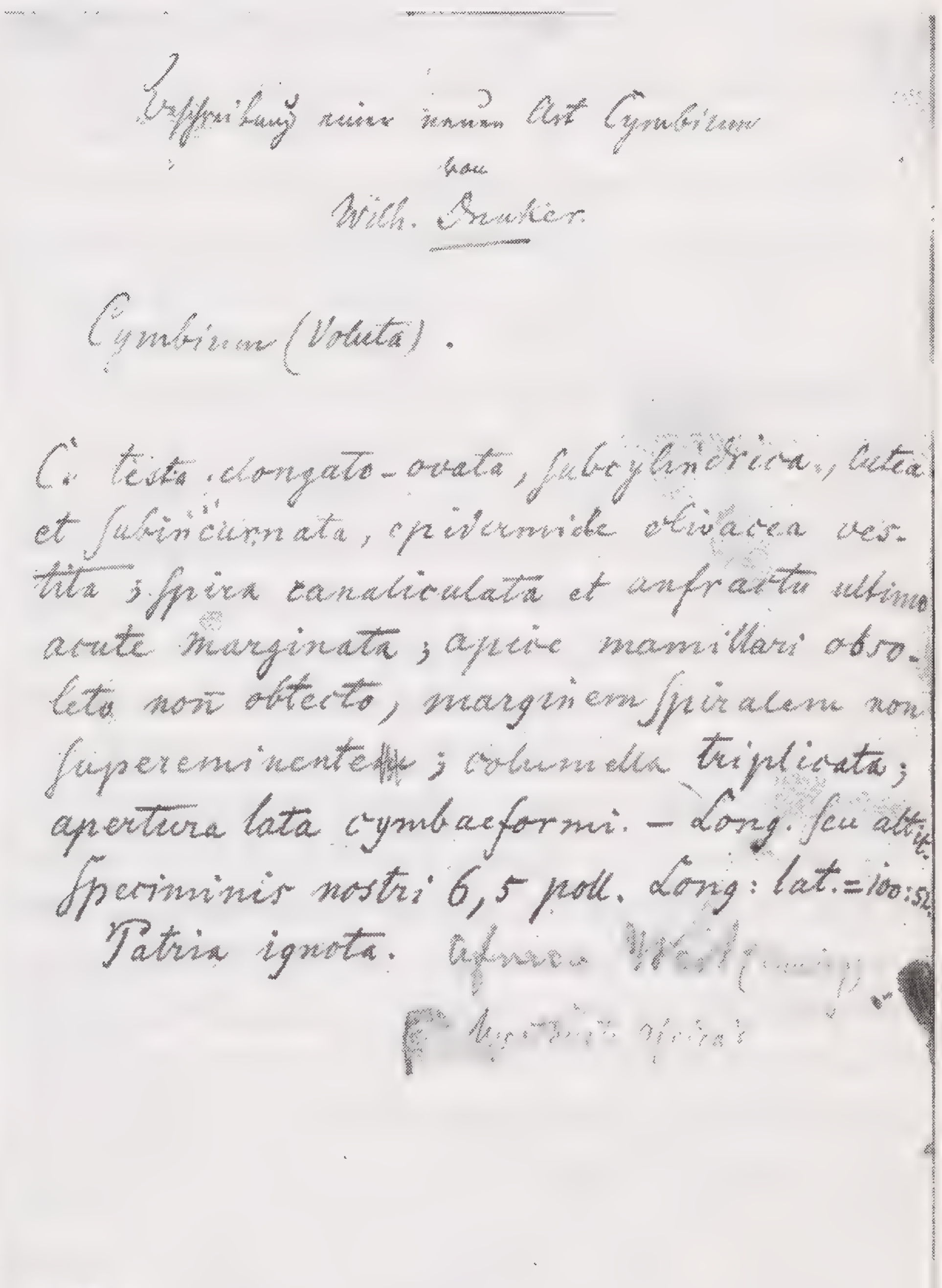


Beschreibung einer neuen Art Cymbium
von
Wilh. Dunker.

Cymbium (Voluta).

C. testa elongato-ovata, ſubcylindrica, lutea et ſubincarnata, epidermide olivacea vestita; ſpira canaliculata et anfractu ultimo acute marginata; apice mamillari obsoleto non obtecto, marginem ſpiralem non ſupereminentem; columella triplicata; apertura lata cymbaeformi. – Long. ſeu alt. ſpeciminis noſtri 6,5 poll. Long: lat.=100:5
Patria ignota. Africa [illegible]
[illegible]

2

FIGS. 1, 2. Wilhelm Rudolph Bernard Hadrian Dunker (1809–1885). FIG. 1: Portrait of Dunker. Museum für Naturkunde der Humboldt-Universität zu Berlin Historische Bild- u. Schriftgutsammlungen Signatur: B I/30; FIG. 2: A sample of Dunker's handwriting. Handschriftenarchiv der malakologischen Sammlung, Museum für Naturkunde.

and co-edited six volumes with Karl Alfred von Zittel (b. 1839 – d. 1904). He maintained correspondence with prominent geologists and paleontologists, including Christian Leopold Freiherr von Buch (b. 1774 – d. 1853) in Berlin (Glaubrecht, 2004) and Gideon Algernon Mantell (b. 1790 – d. 1852) in London.

In 1854 Dunker moved from Kassel to Marburg, where he served as a professor and a director of the mineralogical-geological institute at the Philipps University. The institute, however, did not have an extensive collection, and Dunker used his private collection in his lectures that were often held at his residence. By that time, Dunker's shell collection grew large, partly through purchases, but largely due to gifts by friends, colleagues, and former students. Dunker's reputation as a distinguished conchologist grew along with his collection and soon material from all over the world was sent to him. Dunker rarely left Marburg after 1860 due to an aggravating chronic lung illness. In 1867–1868 he was commissioned to re-curate the shell collection in Naturhistorischen Museum in Hamburg, which he did according to the system of the Adams brothers (H. Adams & A. Adams, 1853–1858) (Weidner, 1993: 111, 133).

Dunker exchanged specimens with other conchologists, most notably Hugh Cuming (b. 1791 – d. 1865) of London, who was one of the most important collectors and dealers of the nineteenth century (Dance, 1980, 1986). Karl Eduard von Martens (b. 1832 – d. 1904), the director and curator in charge of the invertebrate collections at the Berlin für Naturkunde in Berlin, explicitly commented on the Dunker-Cuming correspondence in an unpublished report on the Dunker shell collection (Martens, 1885). According to Martens, Cuming considered Dunker's collection to be the best in Germany and repeatedly visited Dunker in Kassel and in Marburg, possibly upon his return from the Philippines in 1840 or, more likely, in the 1850s, when Dunker's reputation expanded beyond German borders. Although speculative at present, Cuming might have been become acquainted with Dunker through Pfeiffer, whom Cuming met during his trip to Europe in 1832–1835 (Dance, 1980: 484). Surprisingly, Dance mentions Dunker neither in his biography of Cuming (Dance, 1980), nor in his extensive treatment of the history of conchology (Dance, 1966). The second revised edition of the latter work merely lists Dunker's name in the list of Cuming's correspondents (Dance, 1986: 123).

Wilhelm Dunker passed away on March 13, 1885, in Marburg at the age of 77. He published more than 70 scientific publications, 47 of which are dedicated to extant molluscs (Koenen, 1885: 336–338, provides Dunker's bibliography). Many of his papers were published in the *Zeitschrift für Malakozoologie*, the *Journal de Conchyliologie*, the *Proceedings of the Zoological Society*, and the *Philosophical Transactions of the Royal Society of London.* Among Dunker's much acclaimed malacological works are the *Index molluscorum Guinaea* (Dunker, 1853), the *Index molluscorum maris Japonici* (Dunker, 1882), and several taxonomic monographs (Küster, Dunker & Clessin, 1841–1886; Dunker, 1872–1880, 1880–1883) published in the second edition of the *Systematisches Conchylien-Cabinet von Martini und Chemnitz* (Küster et al., 1837–1920).

Among chief contributions by Dunker is publication of the first book devoted exclusively to mollusks of Japan (Dunker, 1861), which followed his earlier paper on the subject (Dunker, 1859). The shells upon which Dunker's lavishly illustrated monograph was based, were collected by the physician Nuhn in the Nagasaki region during his stay in Dejima as a member of a German ship's crew. Nuhn presented his collection to Heinrich Georg Bronn, a professor of zoology at Heidelberg University. Bronn, in turn, loaned this material to Dunker (Cosel, 1998). These works catalyzed subsequent extensive work on the Japanese malacofauna by Carl Emil Lischke (b. 1813 – d. 1886), consisting of three volumes (Lischke, 1869–1874), the first of which was dedicated to Dunker (Cosel, 1998). Much of the Bronn collection, including types of Dunker and Lischke, are housed at Naturmuseum Senckenberg, Frankfurt/Main (Janssen, 1993; Cosel, 1998). Dunker subsequently synthesized the works of others and his earlier works on Japanese mollusks in the *Index molluscorum maris Japonici* (Dunker, 1882).

SOURCES OF SPECIMENS

In preparing the monograph on *Avicula*, Dunker had extensively consulted several collections other than his own. Consequently, some of Dunker's type specimens were – and presumably still are – contained in these collections.

Museum acronyms. BMNH – The Natural History Museum, London; formerly the British Museum (Natural History); GSUB – Geowissenschaftliche Sammlung, Universität Bremen; LMD – Aquazoo-Löbbecke Museum, Düsseldorf; MCZ – Museum of Comparative Zoology, Harvard University; MNHNCU – Museo Nacional de Historia Natural, Havana, Cuba; MNHS – Museo Nacional de Historia Natural, Santiago de Chile; SMF – Naturmuseum und Forschungsinstitut Senckenberg, Frankfurt/Main; ZMA – Zoologisch Museum, University of Amsterdam; ZMB – Museum für Naturkunde, Humboldt University, Berlin; formerly Zoologisches Museum Berlin).

Other abbreviations used in the text: juv, juvenile; LV, left valve; RV, right valve; var, variety.

The Dunker collection. Dunker's shell collection is housed at the Musem für Naturkunde (ZMB) in Berlin. After Dunker died in 1885, his collection of minerals and fossils remained at the University of Marburg in Hesse. Shortly after her husband's death, Dunker's widow offered the shell collection to the Prussian government for the sum of 18,000 marks. Karl Eduard von Martens, the director and curator at ZMB, was appointed to provide a report on Dunker's shell collection. In a letter dated by December 13, 1885 (archived at the *Geheimes Staatsarchiv Preussischer Kulturbesitz, Berlin-Dahlem*), addressed to Dr. von Goßler, the Prussian minister of education and science, Martens gave a detailed account of the collection. He stated that it contained over 119,000 specimens and approximately 26,000 species. It is noteworthy that the much-acclaimed Cuming collection, when purchased by the British Museum of Natural History in 1866, contained 83,000 specimens (Dance, 1980: 495). Martens had also remarked that Dunker left no catalogue of his collection but his former assistant, Dr. Brockmeyer, had compiled a partial inventory that included cephalopods, marine gastropods, and sections of marine bivalves, totalling 33,647 specimens corresponding to 4,746 species. Martens estimated that the remaining part, consisting mainly of land and freshwater mollusks, and the rest of marine bivalves, comprised approximately 85,470 specimens corresponding to 21,586 species. The next year, following Martens' recommendation, Dunker's shell collection was purchased by ZMB.

The Dunker collection was an enormous addition to the ZMB holdings of mollusks. Its scientific value was far beyond just a sheer number of specimens: the collection contained important type material, rare species, well-preserved specimens, lots containing growth series, and comprehensive geographical samples. Moreover, most specimens had good records of locality and collector data. There is no evidence that any shells from this collection were sold or transferred to any other repository after Dunker's death. The shells are typically well preserved and most lots contain original labels written by Dunker (frequently on green paper slips). Dunker's handwriting was authenticated with the help of the manuscripts and correspondence archived at ZMB (Fig. 2). Prior to the present investigation, some type specimens were isolated by Martens, whose hand-written labels contained species names (frequently omitting generic names; Fig. 6) and typically bear his symbol, a cross with a dot in each of the four quadrants, which marks type material (Fig. 6). Typically, Dunker's specimen lots, whether type or not, include printed labels ("coll. Dunker"; Fig. 7) that suggest that these shells were once a part of the Dunker collection.

Museum für Naturkunde, Berlin (ZMB). On a few occasions Dunker mentioned specimens from "Musaeo Berol." (or "Berolinensi"), which shows that he had access to the ZMB malacological collection. This museum was only one of initially three natural history collections of the Berlin University that were housed in the main university building at the time. Its origin predates the foundation of the university in 1810. The rapid growth of the collections soon required a larger space. Eventually, in 1889 the newly established Museum für Naturkunde consolidated individual natural history collections. It has since been among the most important institutions of its kind worldwide. The malacological collection was curated by influential scientists of their times, such as Franz Hermann Troschel (b. 1810 – d. 1882), Karl Eduard von Martens, Johannes Thiele (b. 1860 – d. 1935), and Bernhard Rensch (b. 1900 – d. 1990). When Dunker's collection was incorporated into the museum in 1886, it constituted approximately half of the mollusk holdings. Today, ZMB houses over nine million specimens of mollusks that represent approximately 55,000 described species. Types are estimated to represent roughly 10,000 species. Much of the material originated from private collections, as well as from large scientific expeditions, such as the German Deep Sea Expedition (1898–1899), the German South Pole Expedition (1901–1903), and the Sunda Expedition (1927).

The Scheepmaker collection. Gerrit Scheepmaker (b. 1811 – d. 1854), a school teacher and amateur collector from Amsterdam, made a large donation of shells to the Royal Zoological Society *Natura Artis Magistra* ("Artis"), the precursor of the Zoologisch Museum of the University of Amsterdam (ZMA), in 1844. Many of the specimens were bought in 1855 by Jacob Voorhoeve (b. 1811 – d. 1881), whose collection was offered for sale at an auction in 1872 (Benthem Jutting, 1939: 212; Engel, 1939: 312; Dance, 1986: 154). Some specimens purchased at the sale went directly to Artis and some others came there in later years. In the 1860s, Carl Heinrich Wilhelm Theodor Löbbecke (b. 1821 – d. 1901) purchased the most valuable part of Scheepmaker's collection, which together with other collections acquired by Löbbecke (most notably those of Lischke, van Lennep, and Gruner) became a part of his private conchological museum *Museum Loebbeckeanum* in his house in Düsseldorf. In October 1901, after Löbbecke's death, his widow donated the collection and library to the town of Düsseldorf. The collection was eventually transferred to the newly established municipal *Löbbecke-Museum, Naturkundliche Sammlung der Stadt Düsseldorf*, which opened in 1904. The Löbbecke-Museum was the foundation of today's Aquazoo-Löbbecke Museum (LMD) (Kobelt, 1904; Dance, 1986: 157, 217, 225; Cosel, 1998; Boscheinen, 2004). Thus, presently many Scheepmaker's specimens are either at ZMA or LMD. Even though there is no direct evidence of Dunker's visiting the Scheepmaker collection, the fact that Dunker received specimens from Scheepmaker (Dunker, 1872: 5) indicates that he had examined them prior to the publication of his monograph.

The Gruner collection. Erich Christian Ludwig Gruner (b. 1786 – d. 1857) was a major in the Danish service stationed on St. Thomas Island in the West Indies (now part of the United States Virgin Islands) (Weinder, 1993: 87, 88). Upon retirement, Gruner moved to Bremen in 1827, where his brother, August Wilhelm, owned a West Indies trading company. In 1835 Gruner became a consul and a trading agent for the Grand Duchy of Mecklenburg-Schwerin. His large collection, known as the "Museum Gruneri," was available for study by specialists, including Dunker and Israel Heymann Jonas (b. 1795 – d. 1851). Upon Gruner's death in 1857, a 49-page sale catalog (*Verzeichniß der Conchyliensammlung des verstorbenen Herrn Consul Gruner, welche im Ganzen verkauft werden soll von Bunsen Hausschild 1857.* Bremer Druck 1857) was published, and the collection was advertised for sale in the same year. The collection was intended to be sold as a whole (Tomlin, 1942: 30; Dance, 1986: 157), but it was eventually disseminated in parts. Most specimens were purchased by Hermann von Maltzan (b. 1843 – d. 1891) and later sold to Löbbecke, whose collection is presently housed at LMD (Zilch, 1967). Gruner also exchanged material with colleagues and institutions, and some of his specimens are also extant in other museums, including ZMB and SMF. One of Dunker's type specimens (*Avicula straminea* Dunker, 1852; ZMB 112.865), which originated from the Gruner collection, had a label of the Paetel collection. This suggests that some of Gruner's shells might have been acquired by Friedrich Paetel (b. 1812 – d. 1888). Other specimens might still be extant at the Geowissenschaftliche Sammlung, Universität Bremen (GSUB). Trew (1990: 74) mistakenly attributed the sale of Gruner's collection in 1857 to a different (possibly unrelated) Gruner, a lieutenant general from Denmark, whose cabinet with nearly complete collection of Danish and Norwegian shells, was referred to by Regenfuss (1758) (Dance, 1986: 186).

The Rodatz collection. Little is known about Rodatz and his collection, an important source of material used by Dunker. Hans Albert Rodatz (sometimes referred to as "Rodaz") of Bremen was a ship captain and worked for the Wm. O'Swald & Co. trading company in Hamburg (Weidner, 1993: 87, 88, 91; Schneppen, 2006: 21, 25). He participated in a number of commercial expeditions from Germany to Zanzibar and Western Africa in the period of 1843–1851. Presumably, Rodatz obtained some of the shells during these voyages. He collected many mollusks from the Red Sea in 1845 and offered his specimens to Naturhistorischen Museum in Hamburg, where this material was described by Jonas (1846) (Weidner, 1993: 87, 88). Some of Rodatz's materials collected in Zanzibar were owned by Pfeiffer (Mead, 1995: 259).

The Cuming collection. The collection of Hugh Cuming (b. 1791 – d. 1865) is considered to be one of the largest and taxonomically most significant shell collections of the nineteenth century (Dance, 1980, 1986). Most specimens from the Cuming collection are presently at The Natural History Museum in London (BMNH; Dance, 1986: 209), including the pteriid shells that formed the basis of Reeve's revision of

Avicula (Reeve, 1857). Cuming extensively exchanged shells with and sold specimens to other collectors. For example, the SMF and ZMB collections contain shells that were either obtained from Cuming directly or as part of other collections – Neubert & Janssen (2004) and Köhler (2007) provide examples. Dunker extensively corresponded with Cuming (Martens, 1885) and had an opportunity to examine his collection personally during his stay in London (Dunker, 1872: 5). None of Dunker's pteriid names were based exclusively on the Cuming material, but Dunker illustrated many Cuming's shells. One lot containing two pairs of *Pteria* is accompanied by a label written by Dunker that states that the lot originated from the Cuming collection ("Avicula / England. / H Cuming").

The Tams collection. Little information is available about the collection of Georg Tams, a surgeon and an amateur shell collector. According to Dunker (1853: iii, iv), Tams collected rare shells in Venezuela and Guinea, and made them available for study to other malacologists, such as Pfeiffer and Karl Theodor Menke (b. 1791 – d. 1861). In his introduction to an illustrated catalog of Tam's shells from Guinea, Dunker (1853) highly praised Tams for his enthusiastic and meticulous collecting. Despite the use of academic Latin, the tone suggests that Dunker and Tams were on friendly terms. In 1845 Tams published an account of his travels to southwestern Africa as a medical doctor to the Portuguese commercial expedition (Tams, 1845). This work was translated into English in the same year. Many shells in the Dunker collections – including type and non-type specimens – originate from the Tams collection.

The Gundlach collection. Johannes Christoph Gundlach (b. 1810 – d. 1896), an ardent naturalist and collector, received a degree in liberal arts from the university in Marburg in 1837, where he earned a doctoral degree in zoology in 1838 (Weidner, 1993: 38, 39). On November 1 of the same year he sailed on a voyage to Cuba in a company of Pfeiffer, a malacologist, and C. F. Eduard Otto (b. 1812 – d. 1885), a botanist. Gundlach settled in Cuba, where he continued collecting throughout his life and supplied his European colleagues with specimens from the Caribbean (Ramsden, 1915; Papavero, 1971: 181–183; Weidner, 1993: 39). There is no evidence that Gundlach was in direct contact with Dunker, but his list of correspondents included Louis Pfeiffer, who received specimens of over 200 new molluscan species from him (Jacobson, 1972: 38) and knew Dunker personally. Dunker (1879: 41), however, referred to Gundlach as a friend, suggesting they might also have known each other. Also the fact that Dunker's collection contains lots of shells from Gundlach suggests that both men were in touch. From 1849 on Gundlach repeatedly traveled to Cuba and between 1873–1881 to Puerto Rico. In 1867 he exhibited parts of his collection at the world exhibition in Paris and visited the museum in Berlin. In 1892, he sold his collections to the Spanish government, forming the basis for the Museo Nacional de Historia Natural in Havana (MNHNCU) (Ramsden, 1915: 255; Piechocki, 1992). Specimens from the Gundlach collections are presently housed at the Instituto de Ecología y Sistemática de La Habana, Cuba (Fernández Milera et al., 2002). Most certainly, the specimens used by Dunker were from the European collections. Some of Gundlach's specimens found their way into the collections of ZMB and SMF. ZMB holds a significant number of Gundlach's lots that were obtained from him directly or through Pfeiffer or Dunker.

The Reents collection. No information was found on Reents from Hamburg [referred to as "Reents Hamburgensis" by Dunker (1872: 10)] or the history of his collection. According to archival materials, Reents donated specimens of armadillos to Naturhistorischen Museum in Hamburg in 1868 and, possibly, worked there in the collection of mammals (Weidner, 1993: 214).

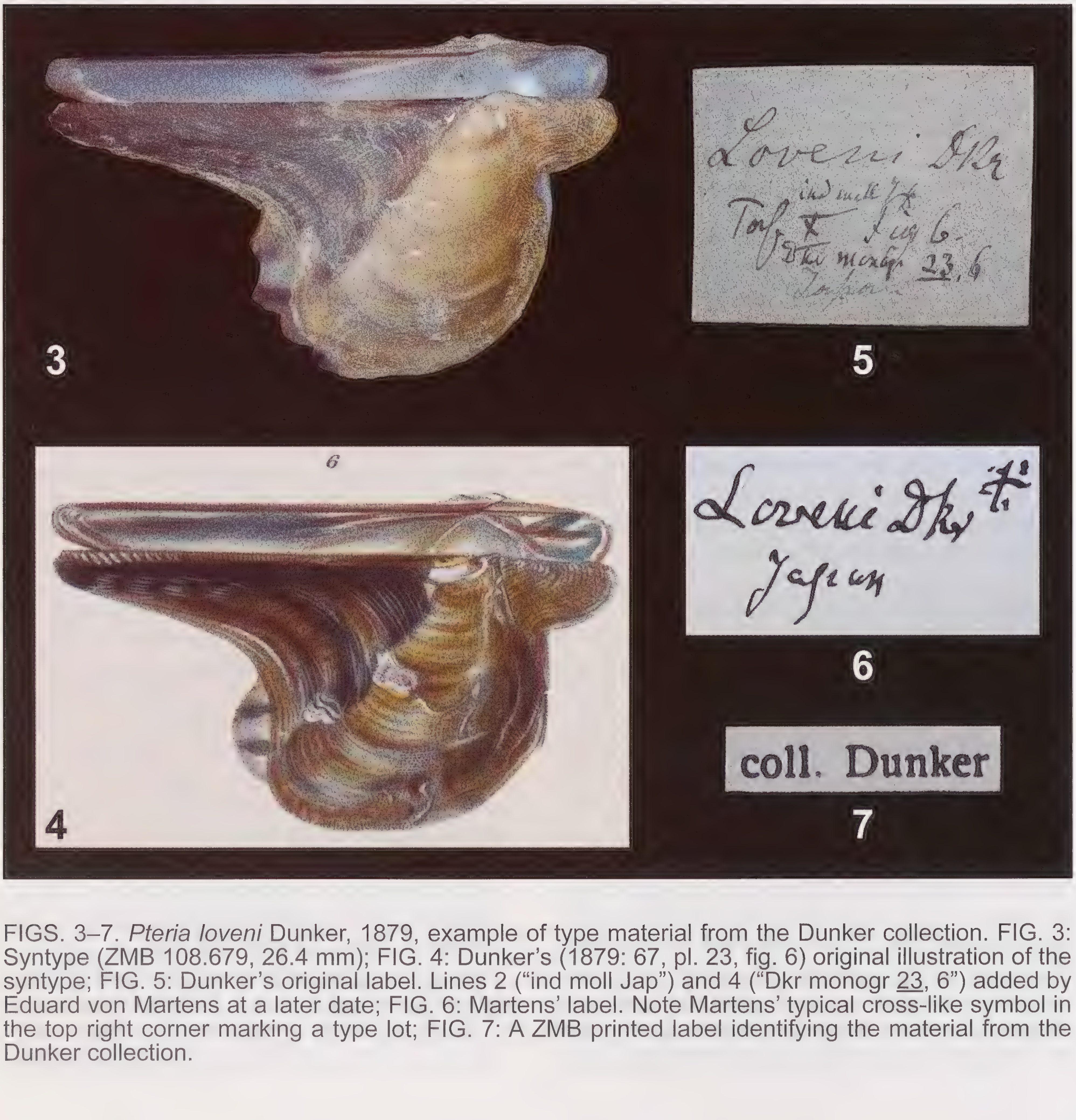
BIBLIOGRAPHICAL SOURCES

Dunker had access to all essential historical and current literature on the genus *Avicula* and produced a sound taxonomic revision, critically pointing out problems in prior taxonomic practice and difficulties in determining species boundaries. He established comprehensive synonymies and resolved many nomenclatural issues. Dunker was well versed in pre-Linnaean literature and cited such ancient writers as Rondelet (1554–1555) and Aldrovandi (1606), among others, and was aware of rather obscure publications of taxonomic significance, such as the *Museum Boltenianum* (Röding, 1798).

Although Dunker was acquainted with foreign collections, such as that of Cuming, he apparently had not seen specimens from Lamarck's collection. The evidence for this is that some of Lamarck's original specimens correspond to shells in the Cuming collections in all respects, and certainly their similarity would not escape the eye of an experienced conchologist. However, Lamarck's work had not been illustrated and his descriptions did not provide enough detail to establish species identity without ambiguity. Dunker regretfully commented on Delessert's (1841) failure to illustrate many of Lamarck's shells, the identity of which could not be established without viewing the original specimens or good illustrations (Dunker, 1880: 76, 77). Thus, in reference to Lamarckian names Dunker relied on the opinion of Deshayes but expressed his skepticism with regard to their identity. Concerning pteriid species described by A. A. Gould (1850, 1851), Dunker concluded that the identity of his species could not be verified in the absence of illustrations or original specimens (Dunker, 1880: 79). Apparently, he was not aware of Gould's atlas with revised descriptions and well-executed figures (Gould, 1852). Therefore, Dunker relied on Reeve's (1857) interpretations of Gould's species and reproduced most of Reeve's figures to illustrate the species named by Gould.

ILLUSTRATIONS

The illustrations of shells in Dunker's monograph (1872–1880) were masterfully executed in color and in all respects are comparable to, if not more exquisite, detailed, and accurate than



FIGS. 3–7. *Pteria loveni* Dunker, 1879, example of type material from the Dunker collection. FIG. 3: Syntype (ZMB 108.679, 26.4 mm); FIG. 4: Dunker's (1879: 67, pl. 23, fig. 6) original illustration of the syntype; FIG. 5: Dunker's original label. Lines 2 ("ind moll Jap") and 4 ("Dkr monogr 23, 6") added by Eduard von Martens at a later date; FIG. 6: Martens' label. Note Martens' typical cross-like symbol in the top right corner marking a type lot; FIG. 7: A ZMB printed label identifying the material from the Dunker collection.

those in *Conchologia Iconica* (Reeve & Sowerby, 1842–1878), one of the most extensive nineteenth century iconographies that is highly praised for its faithful figures. Dunker himself was responsible for preparing most of the illustrations but some were executed at an earlier date by J. Rehbein from Cassel (Dunker, 1872: 5). Most images represented shells from various collections examined by Dunker, whereas others were accurately copied from various sources, most notably from the important iconographies *Neues systematisches Conchylien-Cabinet* (Chemnitz, 1785) and *Conchologia Iconica* (Reeve, 1857). The footer of the plates 1–11 bears an inscription "T. de Suplicy Nbg," possibly referring to the printer; the rest of the plates are free from any such indications. Two plates entitled "*Miess-Müscheln. Mytili.*" from the *Neues systematisches Conchylien-Cabinet* (Chemnitz, 1785: pls. 80, 81) were reproduced by Dunker in their entirety (and in the same order as in the original publication) as the last two plates of *Die Gattung Avicula* (Dunker, 1880: pls. 26, 27).

Rather then being generalized abstractions, Dunker's illustrations were finely detailed and faithfully colored, reflecting the peculiarities of actual specimens, thus alleviating the difficulty in matching actual specimens to the illustrations (Figs. 3, 4). Damages or other irregularities intrinsic to shells were precisely reproduced, whereas foreign objects attached to specimens (such as encrusting barnacles or bryozoans) were omitted. In addition, the shells were depicted in their actual size allowing direct comparison between specimens and illustrations, which is useful when dimensions are not provided in the text. Most minor discrepancies between the illustrations and the specimens are due to slight mismatch in color and marginal damage (often extensive) of the shells, resulting from aging of the paper and handling of the specimens.

Most illustrations followed a convention of depicting the exterior of the right valves with the hinge line at the top and placed horizontally, so that the shells were facing to the right side. The shells were usually shown articulated, so that only a small part of the interior surface of anterodorsal margin of the left valve could be observed. Some shells were depicted disarticulated in a staggered fashion, so that the left valve, while still largely concealed by the right valve, was shifted upward, forward, or diagonally (both upward and forward), providing a clearer view of the interior of the anterior auricle, the hinge, and the color of the nacre along the anterior or dorsal margins. A few images showing shells facing left suggest that the right valve was not available. This supposition is consistent with the observation that in cases where only the left valve was depicted, there was only a single corresponding left valve was present in the Dunker collection. Several specimens were figured with the byssus, which was also found together with the actual specimens.

The choice of whether the shell was shown with articulated or separated valves was probably dictated by the need to show some aspects of shell interior in some specimens, rather than emphasizing whether the valves where actually joined or not. For example, the earlier image of *Avicula loveni* Dunker, 1879 (Fig. 4; Dunker 1879: pl. 23, fig. 6) showed a disarticulated shell with the left valve shifted upward, whereas the same shell was shown articulated in a later publication (Dunker, 1882: pl. 10, fig. 6). In our comments pertaining to the description of the figures, it is assumed the illustrated shell faces right (unless stated otherwise) and the relationship between valves is simply referred to as "depicted articulated," or "with LV shifted upward (forward, or diagonally)."

ANNOTATED CATALOG OF THE DUNKER COLLECTION

The catalog of pteriid specimens from the Dunker collection consists of two sections. The first section lists all the species of *Avicula* named by Dunker and documents the associated type material from the ZMB and LMD collections. The second section describes the figured non-type specimens from these two collections.

The shell dimensions are provided for the right (typically less convex), valve unless it is missing or severely damaged. Hinge length was measured as the longest distance between the extremities of the anterior and posterior auricles parallel to the straight hinge line. Shell height was measured perpendicular to the hinge line to the most distal point of the ventral shell margin. Shell length was taken parallel to the hinge line (exclusive of the auricles).

Nomenclatural Issues

Dunker did not use terms "holotype," "the type," or equivalent expressions in any of his species descriptions that would fix the holotype by original designation (Article 73.1.1; ICZN, 1999: 79). In a few cases, however, the nominal species-group taxa were explicitly or otherwise unambiguously based on single specimens, rendering the extant type specimens recognized as holotypes fixed by monotypy (Article 73.1.2; ICZN, 1999: 79). Most extant type series consist of single syntypes that closely correspond to the original descriptions and illustrations. Some species-group names were based expressly on multiple specimens, but in the majority of original descriptions the number of specimens in the type series could not be established with certainty. Even though it is probable that the specimens from the latter category were based on single specimens, for the lack of definitive supporting evidence they are treated here as syntypes in accordance with the Recommendation 73F ("Avoidance of assumption of holotype"; Article 73.1; ICZN 1999: 80). In all the instances where type series consisted of multiple syntypes, all the specimens were apparently conspecific and in no case lectotype designations were necessitated in accordance with the Recommendation 74G ("Not merely for curatorial purposes"; Amendment of Article 73.1.1; ICZN, 2003: 263). Occasionally, the definitive correspondence of specimens, original descriptions, and illustrations could not be established, such specimens are discussed as "probable type material." In several cases type material could not be located. In accordance with the Code of Zoological Nomenclature, the capitalization of species-group names (Article 28; ICZN 1999: 32) was removed and necessary corrections of original spellings were made (Article 32.5; ICZN 1999: 39–42), but the original spellings are included in parentheses. The taxonomic revision is beyond the scope of the present work, and no attempt is made here to provide synonymies and determine the current taxonomic status of any of the species names discussed in the present work. Consequently, references accompanying each species entry are restricted to original descriptions, illustrations, and subsequent amendments by Dunker or other authors referring to the actual specimens from his collection.

Following the Recommendation 72E ("Publication of information on labels"; ICZN 1999: 79), all the information contained on historical labels is provided, preserving original format, spelling, and crossed-out content. The handwritten labels have been transcribed (preserving original spelling and punctuation), extensive passages translated into English, and the authorship established based on comparison with archival documents of known authorship. The translations and commentaries are included in the square brackets in the label entries. The references to whether the label inscriptions were in ink or in pencil are made only for instances where these co-occurred on the same label and were attributed to different persons.

SPECIES OF *AVICULA* NAMED BY DUNKER

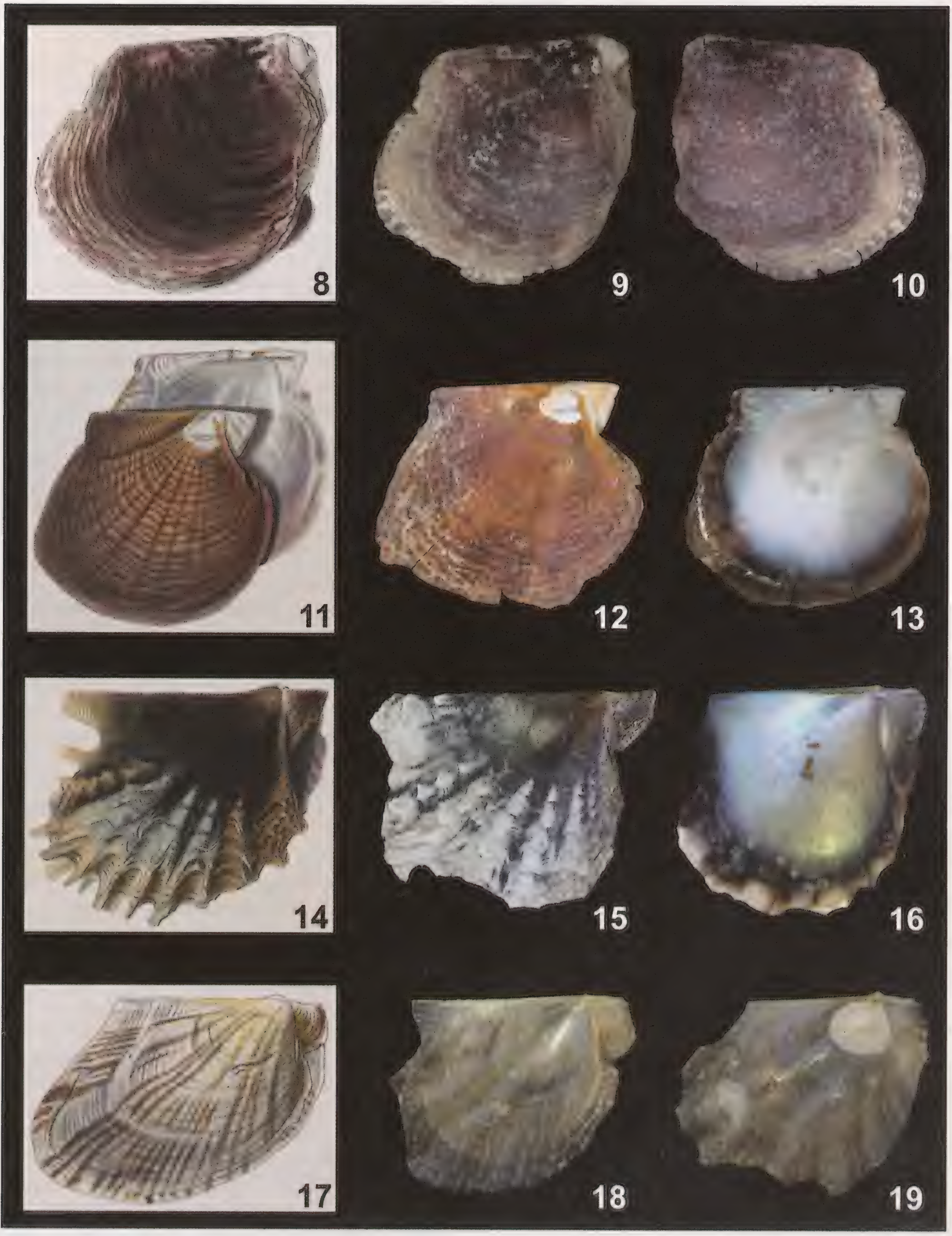
Avicula atrata Dunker, 1872 (Figs. 80–82)

Dunker, 1872: 55, 1879: pl. 19, fig. 1.

Type locality: Unknown.

Probable *syntypes:* ZMB 114.467a, one pair of articulated valves; dimensions (both valves): hinge length 70.4 mm, height 37.4 mm, length 48.7 mm; ZMB 114.467b, single RV; dimensions (RV): hinge length 80.2 mm, height 38.7 mm, length 41.1 mm.

Comments: Dunker's original description unambiguously refers to multiple specimens. One lot from the Dunker collection (ZMB 114.467) contains two very similar, most likely conspecific specimens, one pair of articulated valves and a single RV. The former is very similar to the illustrated specimen in size, overall shape, and color but slightly differs in the shape of the anterior auricle of RV and the pattern of the abraded outer shell layer in the umbonal region. It must be mentioned, however, that the depicted figure is rather generalized and lacks any features to allow establishing a definite match. This specimen matches the figure (Dunker, 1879: pl. 19, fig. 2) that immediately follows the figure of *A. atrata* but it is identified in the text as "*Avicula macroptera* Lamarck." This similarity was previously noted by Martens, who referred to both figures on his label as *A. atrata*. Martens, however, did not include his cross-like symbol to denote these specimens as types. Despite the similarity in the pattern of the fracture of the ventral margin and the color pattern, the figure shows a smaller and articulated specimen. No original Dunker's labels are preserved and, consequently, there are no indications to which species name these specimens correspond and whether they were a part of the type series of *A. atrata*. All labels are in the handwriting of Martens and must have been added later when the Dunker collection had been brought to ZMB. Consequently, for lack of definitive evidence, these specimens are considered as probable syntypes. Four labels accompany the ZMB lot:



FIGS. 8–19. Representative type specimens of *Avicula* from the Dunker collection. Shells arranged with hinge axis oriented horizontally. One of the syntypes is illustrated. Size is expressed as shell length. FIGS. 8–10: *A.* (*Meleagrina*) *atropurpurea* Dunker, 1852; FIG. 8: Dunker's (1872: pl. 3, fig. 3) original illustration; FIG. 9: Holotype, exterior of RV (LMD/LÖB 132905a, 54.3 mm); FIG. 10: Same specimen as Fig. 9, exterior of LV. FIGS. 11–13: *A.* (*M.*) *badia* Dunker, 1852; FIG. 11: Dunker's (1872: pl. 2, fig. 7) original illustration; FIG. 12: Syntype, exterior of RV (ZMB 16383, 76.9 mm); FIG. 13: Same specimen as Fig. 12, interior of LV. FIGS. 14–16: *A.* (*M.*) *bicolor* Dunker, 1880; FIG. 14: Dunker's (1879: pl. 24, fig. 7) original illustration; FIG. 15: Syntype, exterior of RV (ZMB 108.678a, 49.0 mm); FIG. 16: Same specimen as Fig. 15, interior of LV. FIGS. 17–19: *A. cochenhauseni* Dunker, 1872; FIG. 17: Dunker's (1872: pl. 9, fig. 4) original illustration; FIG. 18: Syntype, exterior of RV (ZMB 112.867, 45.6 mm); FIG. 19: Same specimen as Fig. 18, interior of LV.

Label 1: "coll. Dunker" [printed label; affixed to the interior of the single RV, ZMB 114.467b];
Label 2: "*atrata* Dkr" [handwritten by Martens; affixed to the interior of the single RV, ZMB 114.467b];
Label 3: "*atrata* / Dkr / 19. 1. 2" [handwritten by Martens];
Label 4: "cf *semisagitta* / Rv 64" [handwritten by Martens].

Avicula (*Meleagrina*) *atropurpurea* Dunker, 1852 [spelled as "*atro-purpurea*"] (Figs. 8–10)

Dunker, 1852: 76; 1872: 14, pl. 3, fig. 3.
Type locality: Unknown.
Holotype (fixed by monotypy): LMD/LÖB 132905a, one pair of articulated valves; dimensions (RV): hinge length 40.1 mm, height 51.1 mm, length 54.3 mm.
Comments: The description was based upon a unique shell – "quam colore singulare insignis" (Dunker, 1852: 76) – that warrants its designation as holotype by monotypy. The shell derives from the Scheepmaker collection (Dunker, 1852: 76), which, according to the original label, must have been in van Lennep's collection prior its acquisition by Löbbecke. An articulated shell from the LMD/LÖB 132905a lot perfectly matches the shell (likewise depicted articulated) figured by Dunker (1872: 14, pl. 3, fig. 3) in overall shell shape, size, color, and several unique features: (1) the pattern of two dark spots and an abraded part of the umbonal region in RV; (2) the lighter colored ventral and posterior margins relative to the dark chestnut color of the shell surface in RV; (3) a distinctive notch under a weakly sinuated posterior auricle of RV; and (4) the anterior margin of LV slightly protruding anteriorly beyond the anterior margin of RV. Two labels accompany the LMD lot:
Label 1: "v. Lennep 1648 / Avicula / spec. f." [handwritten by Löbbecke];
Label 2: "Avicula / spec. f. / 1 St. v. Lennep [handwriting unidentified] / Löbbecke-Museum" [old printed label of the Löbbecke Museum, c. 1910].

Avicula (*Meleagrina*) *badia* Dunker, 1852 (Figs. 11–13)

Dunker, 1852: 79, 80; 1872: 12, 13, pl. 2, fig. 7.
Type locality: Unknown.
Syntype: ZMB 16383, one pair of disarticulated valves with separated byssus, valves varnished; dimensions (RV): hinge length 55.3 mm, height 65.7 mm, length 76.9 mm.
Comments: The original description mentions the presence of two specimens at ZMB (Dunker, 1852: 80), but only one of the syntypes was located. The shell from the ZMB collection perfectly matches the original description and illustration by Dunker (1872) in size, shape, color, and several unique features: (1) the irregular outline of the eroded outer shell layer in the umbonal region of RV; (2) a short commarginal borehole in the exposed nacre in the umbonal region of RV; (3) the pattern of the remaining fragments of the ligament in the cardinal area of LV; and (4) the irregular outline of the damaged anterior margin ventral to the byssal notch in RV. The shell is illustrated disarticulated with LV shifted diagonally. The posterodorsal margin below the posterior auricle in LV is slightly chipped, while the anterior and ventral margins of RV are substantially damaged. Two radial fractures extend from the ventral margin. These damages were not depicted, suggesting that they had occurred at a later date. Two labels accompany the ZMB lot:
Label 1: "*Meleagrina* / badia Dkr ⁜ / Z. Mal. 1852 p 79 / Dkr Mon 2, 7 / (*Meleagrina crocata* / Swains. Zool. M. / II. tab. 55. / 16383)" [handwriting unidentified; lines 2–4 and catalogue number added by Martens at a later date];
Label 2: "*badia* Dkr ⁜" [handwritten by Martens].

Avicula (*Meleagrina*) *bicolor* Dunker, 1880 (Figs. 14–16)

Dunker, 1879: pl. 24, fig. 7 [figure without name]; 1880: 70.
Type locality: Unknown.
Syntypes: ZMB 108.678a, one pair of disarticulated valves, ventral margin of RV severely damaged; dimensions (RV): hinge length 51.2 mm, height 43.0 mm, length 49.0 mm; ZMB 108.678b, one pair of disarticulated valves; dimensions (RV): hinge length 33.9 mm, height 26.1 mm, length 28.6 mm.
Comments: The species was based on multiple specimens. One of the two shells, ZMB 108.678a, matches the original description and illustration in size, shape, color, and several unique features: (1) the unique pattern of radial tapering black stripes on the surface of RV; (2) the short and wide posterior auricle of RV with the blunt (broken off) distal extremity; and (3) the presence of wide flat scales

predominantly on the posteroventral surface of RV. The shell is depicted articulated. The specimen is markedly damaged along the ventral margin and the dorsal part of the posterior auricle, and some of the scales must have been subsequently broken off the exterior surface of RV. The second specimen in the lot is very similar to the depicted image but differs from it by very light (nearly white) color and a few faint dark colored stripes of uniform width on the posterior half of the RV surface.

Three labels accompany the ZMB lot:

Label 1: "Tab. 24. f. 7.! / *Avicula bicolor* Dkr ⁜ / Vaterland unbekannt [country of origin unknown; here translated]" [line 1 handwritten by Dunker; lines 2 and 3 handwritten by Martens; original label of the Dunker collection];

Label 2: "Mel. *radiata* / Leach / Antill. Mörch" [handwritten by Dunker; label of the Dunker collection];

Label 3: "a. *bicolor* / Dkr" [handwritten by Dunker].

Avicula brevialata Dunker, 1872 (Fig. 113)

Dunker, 1872: 55, 56, 1879: pl. 19, fig. 3; pl. 24, fig. 5 [var.]; 1880: 69 [var.]; 72 [juv.]: pl. 25, fig. 3; 1882: 229, pl. 10, figs. 3–5 [var.].

Type locality: "Habitat ad Amboinas, Molluccenses et Philippinas insulas, pariter et mare Chinense habitare dicitur" (Dunker, 1872: 56).

Type material: Not located.

Comments: Dunker gave more than one locality in the original description, which suggests that the species was based on multiple specimens. However, no shells in the Dunker collection at ZMB decidedly correspond to the single figure (Dunker, 1879: pl. 19, fig. 3) that accompanies the original description (Dunker, 1872: 55, 56), although there are several similar shells present in the collection. Dunker (1879: 56) provided the dimensions for the depicted shell (88 mm length measured between the extremities of the anterior and posterior auricles) and commented on the variability of the extent of the auricles. There are no specimens at ZMB that evidently originate from the Dunker collection with one of the localities given in the description. Therefore, the types are presumably either lost or remain unidentified at ZMB or other collections.

Several specimens of *A. brevialata* and its variety from ZMB were figured by Dunker subsequent to the original description. ZMB 112.862 contains a single pair of joined valves that match the specimen (likewise shown articulated) from Dunker's amendment of *A. brevialata* description ("specimen junius"; Dunker, 1880: 72, pl. 25, fig. 3). The correspondence between the figure and the specimen is evidenced by the outline of the abraded umbo, the slightly distally widening, dark, radial ray running along the depressed area separating the posterior auricle of RV, and the outline of the damaged posteroventral margin of LV. Also, the locality indicated on the label ("Japan") matches the text. The same specimen was also depicted in the *Index Molluscorum Maris Japonici* (Dunker, 1882: pl. 10, fig. 4). The fact that the locality was added subsequently to the original description disqualifies this shell as a probable syntype. One label accompanies ZMB 112.862:

Label: "brevialata Dkr / Ind. Moll. Taf 10 fig 4 / ~~Taf X Fig 4~~. / Dunk mon. Taf 25, fig 3 / Japan." [lines 1, 3, and 5 handwritten by Dunker; lines 2, 4 and crossed-out line 3 (probably in error) handwritten by Martens].

Another lot from the Dunker collection, ZMB 112.861, contains one pair of disarticulated valves that perfectly match the figure of another variety of *A. brevialata* (Dunker 1879: pl. 24, fig. 5) distinguished by an unusual protrusion of the anterior auricles. This is probably a teratological specimen, as evidenced by the presence of encrustation along the dorsal margin of the abnormal auricle that could have caused the abnormal pattern of shell secretion. Two labels accompany ZMB 112.861:

Label 1: "Tab 24. f. 5. [handwritten in pencil by Dunker] / brevialata Dkr var. / Dunk. monogr 24, 5!" [handwritten in ink by Martens];

Label 2: "coll. Dunker" [printed label; affixed to verso of Label 1].

ZMB 114.272 contains two shells that almost certainly represent the specimens of *A. brevialata* (juvenile) and its variety depicted by Dunker (1882: pl. 10: figs. 3 and 5 respectively). The juvenile shell matches the figure in shape, size, color, and, more importantly, in the outline of the abraded region of the umbo and the lighter coloration of the ventral margin and the anterior part of RV due to the remaining periostracum. The shell of the variety, distinguished by its yellowish color (as opposed to the characteristic chestnut color of the rest of the specimens), is similar to the depicted shell in shape, size, color, as well as the pattern of several distinct growth lines and the presence of a radial color pattern along the distal half of the depicted RV. Three labels accompany ZMB 114.272:

Label 1: "*brevialata* / Dkr ⁜ / Japan" [handwritten by Martens];

Label 2: "*brevialata* Dkr / Tab X Fig 3+5 / Japan" [handwritten by Dunker];

Label 3: "Japan!" [handwritten by Dunker].

Avicula (*Meleagrina*) *citrina* Dunker, 1852
(Fig. 70)

Dunker, 1852: 78, 79; 1872: 14, 15, pl. 3, fig. 4; 1880: 71 [var.], pl. 25, fig. 1.

Type locality: Unknown.

Type material: Not located.

Comments: None of the specimens in the Dunker collection at ZMB resemble the illustrations of articulated shells for both *A.* (*M.*) *citrina* and *A.* (*M.*) *citrina* var. Several lots in the Dunker collection at ZMB have similar shells that might have originated from the same source as the type specimens. One lot from Suez, ZMB 112.964, contains a disarticulated shell from the Dunker collection that resembles the articulated shell illustrated by Dunker (1872: pl. 3, fig. 4) in overall shape and light yellow color on most of the outer shell surface of RV. It differs from the illustration by irregular dark purple coloration along the exterior surface of the shell margins and the presence of indistinct radial white rays. Martens identified a disarticulated shell from ZMB 112.826 as "cf. *Avicula citrina* var." This specimen resembles the illustration by Dunker (1872: pl. 25, fig. 1) in overall appearance but differs from it in (1) the shape of the anterior auricle; (2) the green (as opposed to brown) color of the RV exterior; and (3) the absence of distinct fractures and a broken-off piece of the outer shell layer in the middle of RV exterior. Besides, the accompanying label neither indicates the locality, nor contains any evidence that the specimen derives from the Dunker collection. According to Dunker (1872: 14), the specimens upon which the species was based, was from the Scheepmaker collection.

Avicula cochenhauseni Dunker, 1872
(Figs. 17–19)

Dunker, 1872: 27, 28, pl. 9, fig. 4. Note: Dunker (1872: 27) erroneously refers to Figure 3 instead of Figure 4.

Type locality: "Habitat mare Chinense" (Dunker, 1872: 27).

Syntype: ZMB 112.867, one pair of disarticulated valves; dimensions (RV): hinge length 18.7 mm, height 16.2 mm, length 45.6 mm.

Comments: The species upon which the description was based were communicated by Captain Cochenhausen (Dunker, 1872: 27). The reference to the specimens in plural ("specimina") and a range of the dimensions (Dunker, 1872: 27) indicate that the name was based on multiple specimens, one of which was depicted; the other specimen(s) were not located. The depicted shell closely matches the original description and illustration in size, shape, color, and several unique features: the pattern and relative width of radial color stripes and the pattern of commarginal dark-colored stripes. The margins of both valves are severely damaged. The shell is depicted with valves articulated. Possibly due to Dunker's misprint referring to Figure 3 instead of Figure 4, Martens misidentified ZMB 108.690 as a type lot of *Avicula cochenhauseni*. None of the shells in ZMB 108.690 match the original illustration and lack any labels relating it to the Dunker collection. Two labels accompany ZMB 112.867:

Label 1: "coll. Dunker" [printed];

Label 2: "Cap. v. Cochen- / hausen." [handwritten by Dunker; original label of the Dunker collection].

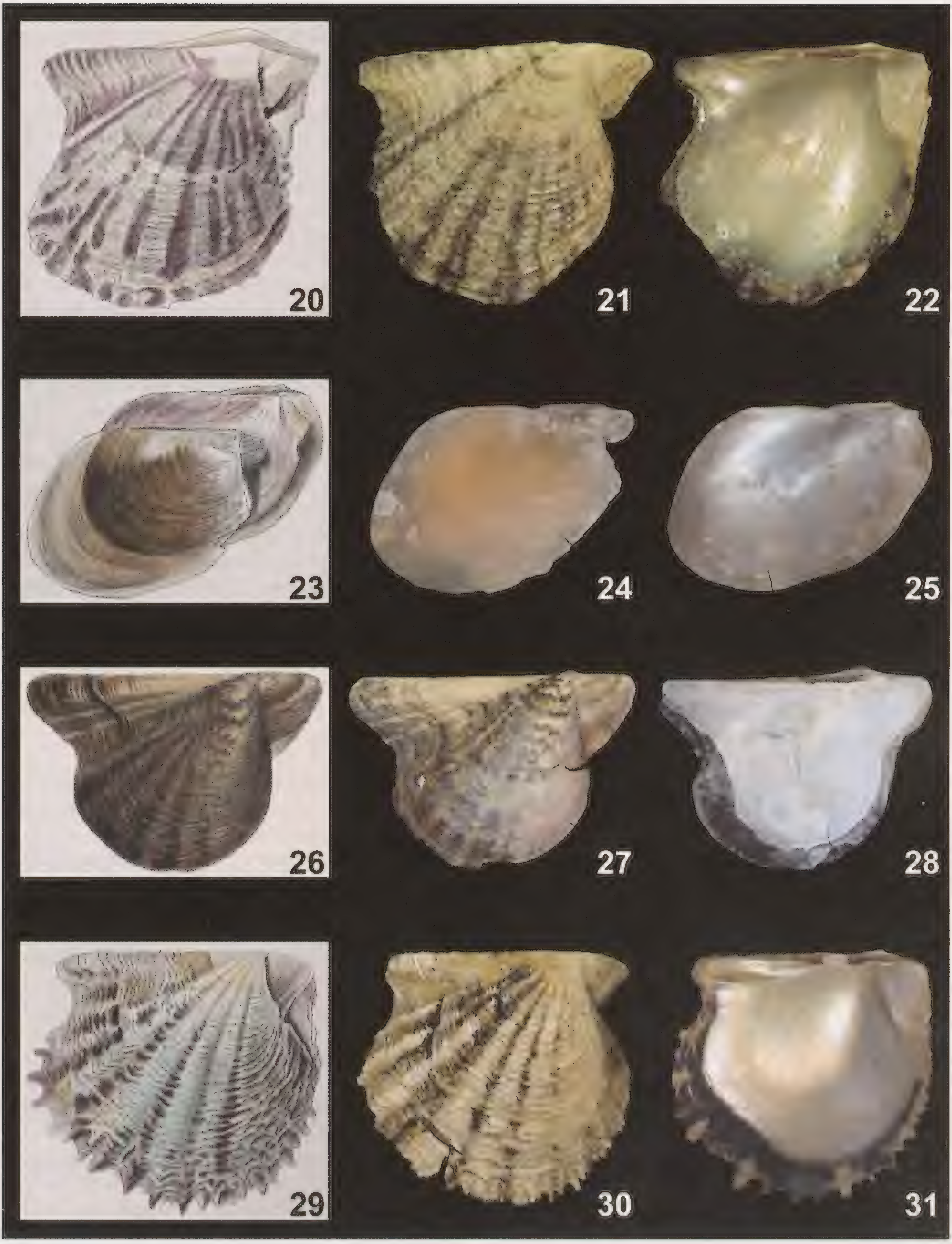
Avicula (*Meleagrina*) *concinna* Dunker, 1872
(Figs. 20–22)

Dunker, 1872: 52, pl. 18, fig. 2.

Type locality: Unknown.

Syntypes: ZMB 108.681a, one pair of disarticulated valves, ventral margin of RV damaged; dimensions (RV): hinge length 38.3 mm, height 34.0 mm, length 32.1 mm; ZMB 108.681b, one pair of disarticulated valves, posteroventral margin of LV severely damaged; dimensions (RV): hinge length 35.8 mm, height 30.5 mm, length 32.2 mm.

Comments: The species was based on multiple specimens. One of the two very similar in appearance shells from the same lot in the Dunker collection, ZMB 108.681a, matches the illustrated articulated shell in shape, size, and color and, more specifically, in (1) the width and pattern of radial dark colored rays on the RV exterior, (2) the short, oblique, dark colored stripes at the posteroventral region of the RV surface, (3) the exposed outline of the anterior margin of LV. Both shells have



FIGS. 20–31. Representative type specimens of *Avicula* from the Dunker collection. Shells arranged with hinge axis oriented horizontally. One of the syntypes is illustrated. Size is expressed as shell length. FIGS. 20–22: *A.* (*Meleagrina*) *concinna* Dunker, 1872; FIG. 20: Dunker's (1872: pl. 18, fig. 2) original illustration; FIG. 21: Syntype, exterior of RV (ZMB 108.681a, 32.1 mm); FIG. 22: Same specimen as Fig. 21, interior of LV. FIGS. 23–25: *A. cornea* Dunker, 1852; FIG. 23: Dunker's (1872: pl. 10, fig. 6) original illustration; FIG. 24: Syntype, exterior of RV (LMD/LÖB 124300a, 34.7 mm); FIG. 25: Same specimen as Fig. 24, interior of LV. FIGS. 26–28: *A. fluctuosa* Dunker, 1880; FIG. 26: Dunker's (1880: pl. 25, fig. 2) original illustration; FIG. 27: Syntype, exterior of RV (ZMB 112.864, 38.0 mm); FIG. 28: Same specimen as Fig. 27, interior of LV. FIGS. 29–31: *A.* (*M.*) *horrida* Dunker, 1872; FIG. 29: Dunker's (1872: pl. 15. fig. 2) original illustration; FIG. 30: Syntype, exterior of RV (ZMB 101.673, 54.7 mm); FIG. 31: Same specimen as Fig. 30, interior of LV.

damaged margins. Three labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed];

Label 2: "*Avicula papi- / lionacea* Lam. / ? / Pfr. / 5." [handwritten by Dunker; label of the Dunker collection];

Label 3: "*concinna* Dkr / ⁜ / mon. 18, 2" [handwritten by Martens].

Avicula cornea Dunker, 1852 (Figs. 23–25)

Dunker, 1852: 74; 1872: 33, 34, pl. 10, fig. 6.

Type locality: Unknown.

Syntype: LMD/LÖB 124300a, one pair of disarticulated valves; dimensions (RV): hinge length 23.6 mm, height 26.4 mm, length 34.7 mm.

Comments: The description was based on multiple specimens, as evidenced by the range of dimensions original provided. A specimen from LMD/LÖB 124300a, that derived from the Scheepmaker collection, perfectly matches the illustration by Dunker (1872: pl. 10, fig. 6) in overall shell shape, size, color, and several unique features: (1) the presence of light-colored encrusted area posterior to the umbo in RV; (2) a commarginal pattern of the abraded area in the umbonal region of RV; and (3) a notch in the margin of the nacreous layer ventral to the anterior auricle in LV. Four labels accompany the LMD lot:

Label 1: "Scheep [handwritten in ink by Löbbecke] / D / habitat cornea / inconnu / ~~membrana~~ / ~~cea Dkr~~" [handwritten in pencil by Dunker];

Label 2: "Scheep [handwritten in ink by Löbbecke] / spadicea / var / Rothes Meer" [handwritten in pencil by Dunker];

Label 3: "Avicula / spadicea Dkr. / Var. / Rotes Meer / 1 St. Scheepm. [handwriting unidentified] / -Museum" [old printed label of the Löbbecke Museum, c. 1910];

Label 4: "Avicula Cornea DKR. / holotyp leg. Scheepmaker / 124300a [handwritten by Boscheinen] / Löbbecke-Museum [printed]."

Avicula coturnix Dunker, 1879 (Figs. 83–85)

Dunker, 1879: 67, pl. 23, figs. 4, 5; 1882: 228, pl. 10, figs. 1, 2.

Type locality: "Mare Japoniae mediterraneum" (Dunker, 1879: 67).

Syntypes: ZMB 108.689a, one pair of articulated valves, ventral margins of both valves damaged; dimensions (both valves): hinge length 37.0 mm, height 11.1 mm, length 22.2 mm; ZMB 108.689b, one pair of disarticulated valves, ventral margins and posterior auricles of both valves damaged; dimensions (RV): hinge length 30.3 mm, height 11.5 mm, length 17.7 mm.

Comments: The species was based on multiple specimens. The articulated shell, ZMB 108.689a, closely matches the original description and illustration size, shape, color, and the unique features of coloration: (1) a noticeably wider spacing between the two most distal dark stripes at the tip of the posterior auricle in LV; (2) a denser and darker colored pattern of brown blotches along the ventral margin, especially at the posteroventral extremity of LV; and (3) a wide commarginal stripe in the middle of RV. In the depicted shell, the margins appear intact, whereas in the actual specimen they are damaged in both valves, but less so in LV. The disarticulated shell, ZMB 108.689b, was illustrated by Dunker in a later publication (1882: pl. 10, figs. 1, 2). The specimen closely resembles the illustration by the pattern of wide, commarginal, arch-like blotches in both valves. In its present condition, the shell is substantially damaged along margins of both valves and the posterior auricles. Five labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed label];

Label 2: "*coturnix* Dkr / mon 23, 3. 4 ! / ind. moll Jap. 10, 1. 2" [handwritten by Martens];

Label 3: "*coturnix* / Dkr ⁜" [handwritten by Martens];

Label 4: "Japan!" [handwritten by Dunker];

Label 5: "Av. pen- / nata Dkr." [handwritten by Dunker].

Avicula cypsellus Dunker, 1872 (Figs. 86–88)

Dunker, 1872: 25, 26, pl. 8, fig. 4.

Type locality: "Habitat in Oceano Indico fide beato Scheepmaker" (Dunker, 1872: 25).

Syntype: LMD/LÖB 132969a, one pair of disarticulated valves; dimensions (RV): hinge length 94.3 mm, height 43.7 mm, length 51.7 mm.

Syntype: The figured shell is from the Scheepmaker collection. Despite the fact that the original description does not mention the presence of multiple specimens and provides the dimensions for the "exemplar depictum" (Dunker, 1872: 25), it does not contain an explicit statement that it was based on a single specimen. LMD/LÖB 132969a contains a disarticulated specimen that perfectly matches the original description and illustration in size, shape, color, and several unique features: (1) the pattern of the abraded outer shell layer at the umbonal region, the anterior auricle, and the anterodorsal part of RV; (2) the shape of unusually protruded anterior auricle; and (3) a light-colored ray separating the valve from the posterior auricle. No original labels accompanied the LMD lot.

Avicula (*Meleagrina*) *echinus* Dunker, 1872 (Fig. 71)

Dunker, 1872: 40, 41, pl. 13, fig. 2.
Type locality: "Habitat in portu ad Habanam" (Dunker, 1872: 40).
Type material: Not located.
Comments: The depicted specimen derives from the Gundlach collection. A reference to "diese kleine Muschel" ("this tiny shell"; Dunker, 1872: 41; here translated) in the original description indicates that the description was probably based on a single specimen, although this assumption could not be verified.

Avicula (*Meleagrina*) *fimbriata* Dunker, 1852 (Figs. 72, 73)

Dunker, 1852: 79; 1872: 13, 14, pl. 3, figs. 2, 6.
Type locality: "Patria America centralis dicitur" (Dunker, 1852: 79).
Type material: Not located.
Comments: The description was based on multiple specimens as evidenced by the range of dimensions provided in the original description. The location of the type material is unknown.

Avicula fluctuosa Dunker, 1880 (Figs. 26–28)

Dunker, 1880: 71, 72, pl. 25, fig. 2.
Type locality: Unknown.
Syntype: ZMB 112.864, one pair of disarticulated valves; dimensions (RV): hinge length 55.4 mm, height 37.8 mm, length 38.0 mm.
Comments: The original description does not contain an explicit statement that it was based on a single specimen and does not include shell dimensions. A single shell present in the type lot closely corresponds to the figure accompanying the original description. The shell matches the illustration in size, shape, color, and two unique features: a large anterior auricle with a distinct pattern of growth accretion separating the surface of the auricle into tree parts and the pattern of commarginal dark stripes, which become more zigzagged in the anterior half of the RV exterior surface. The ventral margin of RV is slightly more damaged than depicted and bears a fracture, extending from the area directly underneath the anterior auricle to approximately the middle of the valve. The original description does not indicate type locality ("patria ignota"). However, most labels (including those in Dunker's handwriting) contain the locality "Australia," possibly due to the specimen's resemblance to the Australian shell described by Reeve (1857, sp. 73, pl. 18, fig. 69) as *Avicula atlantica* Lamarck, 1819, the reference to which also appears on the labels.
Five labels accompany the ZMB lot:
Label 1: "coll. Dunker" [printed label];
Label 2: "*atlantica* Lam / Reeve T. 18. sp. / 73, f. 69 / 'Australia Jukes'" [handwritten by Dunker];
Label 3: "Australia / cf *sterna* A Gould / (*heteroptera* Rv 67" [handwritten by Martens];
Label 4: "Zoologisches Museum Berlin. [printed] / Australien Dunker" [handwriting unidentified; typical ZMB collection label].

Avicula (*Meleagrina*) *grisea* Dunker, 1872 (Fig. 74)

Dunker, 1872: 11, 12, pl. 2, fig. 5.
Type locality: Unknown.
Type material: Not located.
Comments: The original description does not contain an explicit statement that it was based on a single specimen and does not include shell dimensions. The location of the type material is unknown.

Avicula gruneri Dunker, 1872 (Fig. 114)

Dunker, 1872: 51, 52, pl. 18, fig. 1.
Type locality: Unknown.
Type material: Not located.
Comments: The original description does not contain an explicit statement that it was based on a single specimen and does not include shell dimensions. Dunker (1872: 51, 52) indicated that the figured specimen came from the Gruner collection. The location of the type material is unknown and presumed to be either lost or unidentified at the GSUB, LMD, or SMF collections.

Avicula (*Meleagrina*) *horrida* Dunker, 1872 (Figs. 29–31)

Dunker, 1872: 11, pl. 2, fig. 4; 46, pl. 15. fig. 2.

Type locality: “Habitat in litore Venezuelensi prope Cumanam nec non in Antillis insulis” (Dunker, 1872: 11).

Syntype: ZMB 101.673, one pair of disarticulated valves; dimensions (RV): hinge length 48.8 mm, height 49.0 mm, length 54.7 mm.

Comments: The species was based on multiple specimens. The shell from the Gruner collection depicted on Dunker’s pl. 2 was not located. The specimen from the ZMB collection (ZMB 101.673) perfectly matches the original description and the second figure (pl. 15, fig. 2) in size and shape, and, more importantly, in the pattern of purple radial rays and two large dark spots at the posterior part of RV exterior surface. Three labels accompany the ZMB lot:

Label 1: “*Avicula horrida* Dunk / Dunk. Taf. 15 fig 2! p. 46 / Venezuela” [handwritten by Martens];

Label 2: “*horrida* Dkr ⁜” [handwritten by Martens];

Label 3: “Zoologisches Museum Berlin. [printed] / Puerto *horrida* / (Dunker) * / 101 673 / Venezuela” [handwritten by Rudolf Kilias].

Avicula hyalina Dunker, 1852 (Figs. 32–34)

Dunker, 1852: 75; 1872: 32, 33, pl. 10, figs. 3, 4.

Type locality: “Insula Van Diemen; attulit ornat. Schayer” (Dunker, 1852: 75).

Syntypes: ZMB 108.692a, one pair of disarticulated valves, margins of both valves damaged; dimensions (RV): hinge length 16.6 mm, height 16.2 mm, length 20.0 mm; ZMB 108.692b, one pair of disarticulated laves, RV broken, posteroventral margin of LV severely damaged, pieces enclosed; dimensions (RV): hinge length 14.6 mm, height 16.5 mm, length 22.2 mm; ZMB 108.693, two disarticulated pairs and one RV, all specimens severely damaged; dimensions (RV): hinge length 13.1–16.3 mm, height 12.5–17.2 mm, length 19.1–22.2 mm.

Comments: The original description was based on the material from ZMB, as stated by Dunker (1852: 75, “Schayer. Exstat in Museo Berol”). The lot contains the original label that mentions the name “Schayer,” which is also referred to in the original description. However, none of the specimens from the ZMB collection unambiguously match the illustrations. The shells in ZMB 108.692a are very similar to the depicted specimens in shape and size, but the pattern of commarginal color stripes does not match with certainty. It must be noted that the illustrations are quite unspecific and might be not entirely accurate representations of the extant specimens; it is possible that the illustrations are composite images. The same observations hold for the ZMB 108.693 lot. Two label accompany ZMB 108.692a,b:

Label 1 (recto): “*Meleagrina* / *Avic hyalina* ⁜ / Dkr / Z Mal 1852 p. 25 / cf *perviridis* Rv / Zettel lag daneben, / müßte in diese / Schachtel gehören! / Vandiemsland. [with label aside, / probably belongs into this box / Vandiemsland (=Tasmania); here translated] / Schayer.” [lines 1, 2, 3, 9, 10 handwriting unidentified; asterisk and lines 4 and 5 subsequently added by Martens; lines 6–8 subsequently added, handwriting attributed to Rudolf Kilias; original ZMB label];

Label 1 (verso): “~~Meleagrina~~ / ~~spec.~~ / ~~Vandiemsland.~~ / ~~Schayer.~~” [handwriting unidentified].

Label 2: “Tasm. / hyalina Dkr. / ⁜ / Dkr. 10,4” [handwritten by Martens].

One label accompanies ZMB 108.693:

Label: “Meleagrina [handwritten in pencil, attributed to Dunker] / hyalina, Dkr. [handwritten in black ink by Martens] / Vandiemsland Schäyer.” [handwritten in brown ink, attributed to Dunker].

Avicula jamaicensis Dunker, 1879

Avicula cornea Reeve, 1857: Species 73, pl. 17, fig. 73; Dunker, 1879: 65, pl. 22, fig. 10.

Type locality: “Jamaica” (Reeve, 1857).

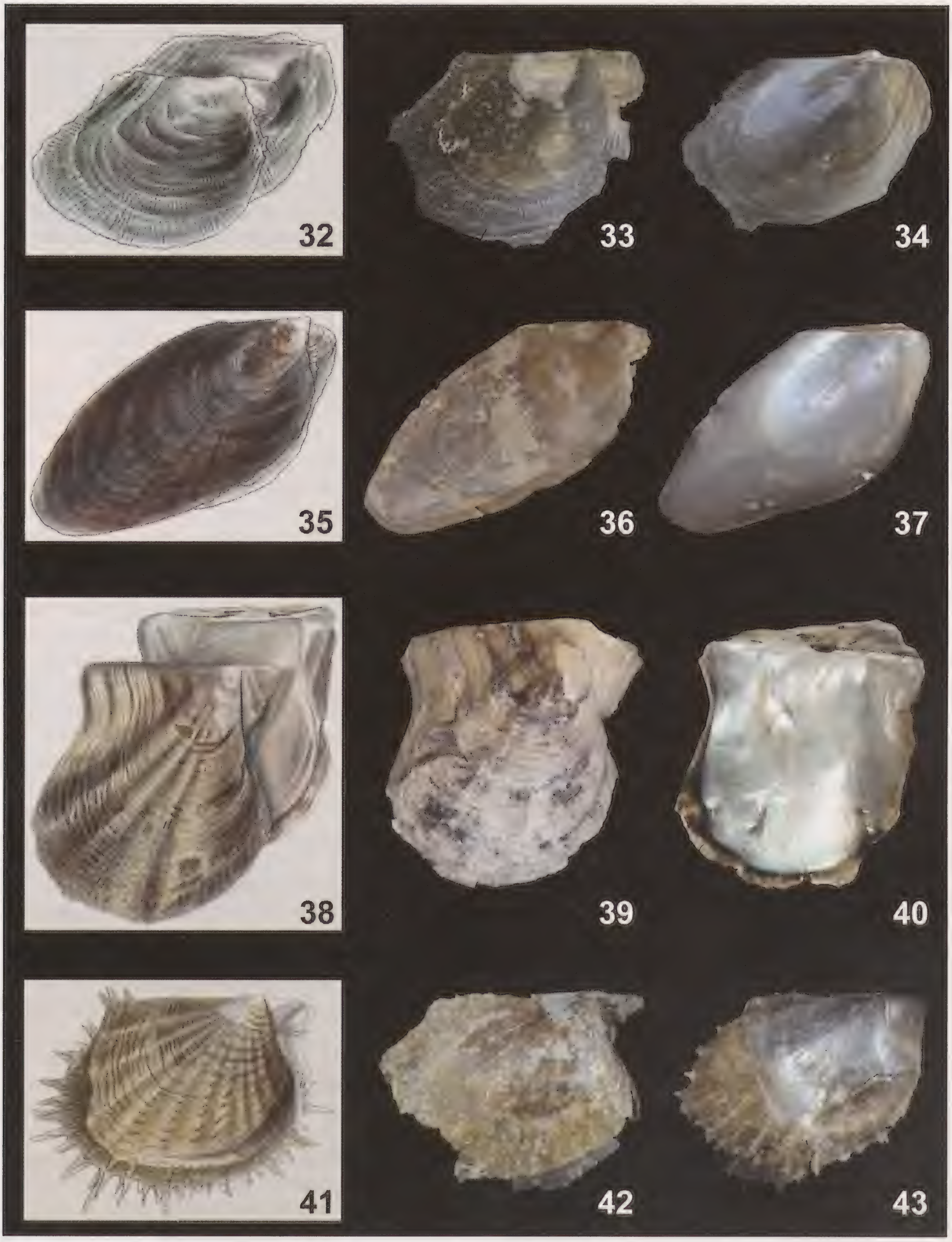
Comments: A. jamaicensis is a substitute name for *A. cornea* Reeve, 1857, a junior primary homonym of *Avicula cornea* Dunker, 1852. The illustration was based on the specimen from the Cuming collection figured by Reeve (1857: pl. 17, fig. 73). The original Reeve figure shows the specimen attached to coral, whereas Dunker did not illustrate the coral.

Avicula japonica Dunker, 1852 (Figs. 35–37)

Dunker, 1852: 73, 74; 1872: 33, pl. 10, fig. 5; 1882: 230.

Type locality: “Haec species incola est maris Japonici” (Dunker, 1852: 74).

Holotype (fixed by monotypy): LMD/LÖB 124280a, one pair of disarticulated valves; dimensions (RV): hinge length 18.1 mm, height 30.0 mm, length 38.9 mm.



FIGS. 32–43. Representative type specimens of *Avicula* from the Dunker collection. Shells arranged with hinge axis oriented horizontally. One of the syntypes is illustrated. Size is expressed as shell length. FIGS. 32–34: *A. hyalina* Dunker, 1852; FIG. 32: Dunker's (1872: pl. 10, fig. 4) original illustration; FIG. 33: Syntype, exterior of RV (ZMB 108.692a, 20.0 mm); FIG. 34: Same specimen as Fig. 33, interior of LV. FIGS. 35–37: *A. japonica* Dunker, 1852; FIG. 35: Dunker's (1872: pl. 10, fig. 5) original illustration; FIG. 36: Holotype, exterior of RV (LMD/LÖB 124280a, 38.9 mm); FIG. 37: Same specimen as Fig. 36, interior of LV. FIGS. 38–40: *A.* (*Meleagrina*) *lichtensteini* Dunker, 1852; FIG. 38: Dunker's (1872: pl. 2, fig. 3) original illustration; FIG. 39: Syntype, exterior of RV (ZMB 16.376a, 46.5 mm); FIG. 40: Same specimen as Fig. 39, interior of LV. FIGS. 41–43: *A.* (*M.*) *longisquamosa* Dunker, 1852; FIG. 41: Dunker's (1872: pl. 2, fig. 6) original illustration; FIG. 42: Syntype, exterior of RV (ZMB 101.674, 35.9 mm); FIG. 43: Same specimen as Fig. 42, interior of LV.

Comments: The phrase "Singularis sane forma" in the original description (Dunker, 1852: 74) and the word "Original" that appears on the labels written by Dunker and Löbbecke warrant the designation of the extant specimen as holotype by monotypy. The shell derives from the Scheepmaker collection that had later become part of the Löbbecke collection (Dunker, 1852: 74; 1882: 230). The shell from the LMD collection closely matches the figure by Dunker (1872: pl. 10, fig. 5) in size, shape, color, and two unique features: a dark spot immediately posterior to the umbo in RV and a small anteriorly protruding part of the anterior margin of RV. The shell is figured articulated. See additional comments on the misuse of the name "japonica" in the entry for *Avicula* (*Meleagrina*) *martensii* Dunker, 1880. Five labels accompany the LMD lot:

Label 1: "Scheepm. 1124 / Avicula / japonica Dunker / Original / Dunker Mart. Chem. / T. 10 f. 5" [handwritten by Löbbecke];

Label 2: "japan / japonica Dk / ~~cornea Dkr~~" [handwritten by Dunker];

Label 3: "japonica Dkr. / Original." [handwritten by Dunker];

Label 4: "Avicula / japonica Dkr. / 1 [handwriting unidentified] Löbbecke-Museum" [old printed label of the Löbbecke Museum, c. 1910];

Label 5: "Avicula japonica DUNKER / 1 Ex. / Vorlage z.d. Abb. i. M. u. Ch. / Bd.VII,3. Taf. 10, Figur 5. [typed] / Löbbecke-Museum" [printed].

Avicula jeffreysii Dunker, 1880 (Figs. 89–91)

Dunker, 1879: pl. 24, fig. 3 [figure without name]; 1880: 69.

Type locality: "Litus Guineense teste mercatore, qui speciem venalem habuit" (Dunker, 1880: 69).

Syntype: ZMB 108.691, one pair of disarticulated valves; dimensions (RV): hinge length 74.2 mm, height 36.8 mm, length 39.5 mm.

Comments: The original description does not contain an explicit statement that the species was based on a single specimen and does not provide the dimensions. ZMB 108.691 contains a single shell that closely corresponds to the figure accompanying the original description in size, shape, color, and two unique features: the irregular pattern of radial and commarginal dark stripes on the exterior of RV and the light-colored S-shaped stripe in the middle of the posterior auricle. The posteroventral part of the valve is broken off but is enclosed with the specimen. Omitted from the illustration is a large encrustation at the proximal part of the posterior auricle of RV. Five labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed label];

Label 2: "Tab. 24. fig. 3." [handwritten by Dunker];

Label 3: "*jeffreysii* / Dkr ⁜ / Taf 24 fig 3 / Guinea" [lines 1, 2 handwritten by Dunker; asterisk and lines 3, 4 handwritten by Martens];

Label 4: "*jeffreysii* / Dkr ⁜" [handwritten by Martens];

Label 5: "78 / *Avicula semi* / *sagitta* / Lam Afrique" [handwriting unidentified].

Avicula (*Meleagrina*) *lichtensteini* Dunker, 1852 (Figs. 38–40)

Dunker, 1852: 77; 1872: 10, pl. 2, fig. 3.

Type locality: "Insulae Sandwich" (Dunker, 1852: 77).

Syntypes: ZMB 16.376a, one pair of disarticulated valves; dimensions (RV): hinge length 49.3 mm, height 56.6 mm, length 46.5 mm; ZMB 16.376b, one pair of disarticulated valves with RV ventral margin severely damaged; dimensions (LV): hinge length 50.0 mm, height 65.6 mm, length 59.4 mm; ZMB 976, soft body in alcohol.

Comments: The description was based on the material housed at ZMB (Dunker, 1852: 77, "Musaeo Berol."; 1872: 10, "Exstat in collectione rerum naturalium regio Berolinensi"). Martens marked ZMB 16.376 as the type lot. One of the two shells, ZMB 16.376a, resembles the illustrated shell in overall shape and the pattern of dark blotches in near-umbonal region. It differs, however, from the depicted shell by having an angle formed by the dorsal margin and the anterior auricles. (The illustration shows the anterior auricle continuous with the straight hinge line.) The second shell from the same lot is similar to the figured shell in the pattern of the remaining part of the ligament in LV and the overall shape of the dorsal half of the shell (the ventral part of RV is missing), but differs from it by the lack of the distinct dark brown blotches (present in the other specimen). It is possible that the illustration is a composite of the two shells from the type lot; however this claim is difficult to substantiate. Another lot, ZMB 976, labeled as "*Avicula margaritifera* from Sandwich Islands, leg. Deppe," contains soft tissues in alcohol that correspond to the shells from ZMB 16.376. Three labels accompany ZMB 16.376:

Label 1: "*Meleagrina* / ⁜ / *lichtensteinii* Dkr / Z. Mal. 1852. p 77 / *A. nebulosa* Conrad / Rv 33 / Sandwichs-Inseln / Deppe / 16376" [lines 1, 3, 6, 7 handwriting unidentified; lines 2, 4, 5, 8, 9 added subsequently by Martens];

Label 2: "Sandwich / *nebulosa* Conr / *lichtensteini* Dkr ⁜" [handwritten by Martens];

Label 3: "Zoologisches Museum Berlin. [printed] / Sandwich Ins Deppe" [handwritten by Rudolf Kilias].

Avicula (*Meleagrina*) *longisquamosa* Dunker, 1852 (Figs. 41–43)

Dunker, 1852: 76, 77; 1872: 12, pl. 2, fig. 6.

Type locality: "Litus Venezuelense ad Porto Cabello" (Dunker, 1852: 77).

Syntype: ZMB 101.674, one pairs of disarticulated valves with byssus attached to RV; dimensions (RV): hinge length 24.9 mm, height 28.2 mm, length 35.9 mm.

Comments: Previously, Mikkelsen et al. (2004) regarded the only specimen in the type lot as holotype. However, the absence of the expressed unambiguous statement that the original description was based a single specimen or any other definitive evidence renders it a syntype. A photograph of the syntype and the corresponding illustration (both shown in the same orientation) were published in color as figs. 2 and 3, respectively, by Mikkelsen et al. (2004: 477).

The specimen was received by Dunker from Tams (Dunker, 1852: 77; 1872: 12). The shell from the ZMB collection perfectly matches the original description and the illustration in size and shape, and several unique features: (1) the presence of the byssus; (2) the outline of the incrusted area posterior to umbo in RV; (3) the pattern of radial light-colored rays; and (4) the shape and the extent of the anterior auricle in RV. A slight discrepancy in the exterior valve surface color between the specimen and the figure is most likely due to the deteriorative changes in both the shell and the paper. The shell is depicted articulated with the byssus produced through the byssal notch. Both valves of the actual specimen are severely marginally damaged and most long marginal scales are broken off, as in the original illustration. According to a label by Rudolf Kilias, LV was broken and repaired. Three labels accompany the ZMB lot:

Label 1: "Avicula. / Porto Cabello. / Dr. G. Tams." [handwritten by Dunker on a green paper slip];

Label 2: "coll. Dunker" [printed; affixed to verso of Label 1];

Label 3 (recto): "Zoolog. Museum Berlin [printed] / Typ.-Mat. [handwritten in pencil] / Holotypus [handwritten in blue ink] / longisquamosa / Dunker [handwritten in pencil] / 101 674 [handwritten in blue ink] / leg. Tams [handwritten in pencil] / Puerto Cabello [handwritten in black ink] / Coll. [handwritten in pencil] Dunker" [handwritten in black ink] [notes in pencil and blue ink handwritten by Ingeborg Kilias; notes in black ink are typical ZMB labels handwritted by unidentified person];

Label 3 (verso): "war gebrochen, / geklebt! [was broken, / glued; here translated] / V. 1972" [handwritten by Ingeborg Kilias].

Avicula loveni Dunker, 1879 [spelled as "*Lovéni*"] (Figs. 3, 4)

Dunker, 1879: 67, pl. 23, fig. 6; Dunker, 1882: 229, pl. 10, fig. 6.

Type locality: "Mare Japoniae mediterraneum" (Dunker, 1879: 67).

Syntype: ZMB 108.679, one pair of disarticulated valves; dimensions (RV): hinge length 57.3 mm, height 28.2 mm, length 26.4 mm.

Comments: The original description does not contain an explicit statement that the species was based on a single specimen and does not provide the dimensions. The single shell in the type lot closely corresponds to the figure accompanying the original description. The shell closely matches the original description and illustration size, shape, color, and several specific details: (1) the distinct, sharply defined pattern of dark coloration of the posterior auricle of RV; (2) the pattern of dark commarginal lines on the exterior of RV; and (3) the presence of dark lines on the inner posteroventral margin of LV. The shell is depicted disarticulated with LV shifted upward. The same shell was illustrated by Dunker in a later publication (Dunker, 1882: 229, pl. 10, fig. 6), but shown articulated and somewhat more generalized. Also, unlike the original illustration, the later figure shows a damaged ventral margin, suggesting that it has been made from the same specimen at a later date rather than being copied from the original figure. Three labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed label];

Label 2: "*Loveni* Dkr / ind moll Jap / Taf X Fig 6. / Dkr monogr 23, 6 / Japan" [lines 1, 3, and 5 handwritten in brown ink by Dunker;

lines 2 and 3 in blue ink were added by Martens at a later date];

Label 3: “⁜ / *Loveni* Dkr / Japan” [handwritten by Martens].

Avicula (*Meleagrina*) *martensii* Dunker, 1880

(Figs. 44–46)

Dunker, 1879: 66 [per erratum *Avicula* (*Meleagrina*) *japonica*], pl. 23, figs. 2, 3; 1880: 79, 81, 84; 1882: 229, pl. 10, figs. 7, 8.

Type locality: “Mare Japonicum” (Dunker, 1879: 66).

Syntypes: ZMB 108.685, one pair of disarticulated valves; dimensions (RV): hinge length 59.2 mm, height 63.3 mm, length 57.9 mm; ZMB 108.683, one pair of disarticulated valves; dimensions (RV): hinge length 58.2 mm, height 62.8 mm, length 60.0 mm; ZMB 108.684a, one pair of disarticulated valves; dimensions (RV): hinge length 49.7 mm, height 48.2 mm, length 43.8 mm; ZMB 108.684b, one pair of disarticulated valves, anteroventral margin of RV damaged; dimensions (LV): hinge length 56.3 mm, height 46.3 mm, length 50.0 mm.

Comments: Dunker originally described and illustrated this species as “*Avicula* (*Meleagrina*) *japonica*,” the name he had established earlier for a different species, *Avicula japonica* Dunker, 1852. Subsequently Dunker expressly admitted the error (Dunker, 1880: 79, 81, 84; 1882: 229) and proposed a replacement name – *martensii* – for the junior primary homonym in the following year (Dunker, 1880: 79).

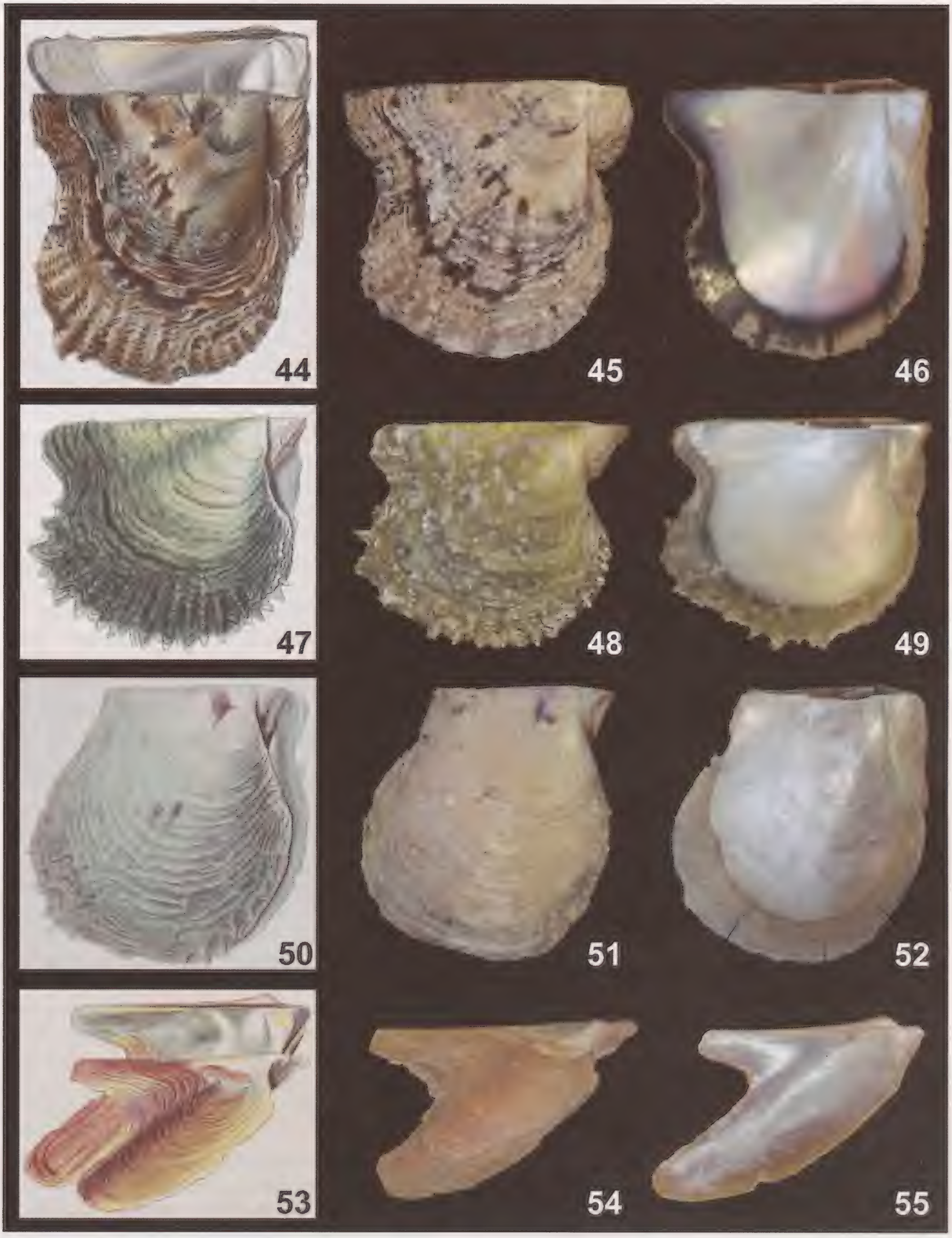
The species was based on multiple specimens. All the extant specimens appear to be conspecific. The shells in ZMB 108.683 and ZMB 108.685 from the Dunker collection contain labels with the phrase “original,” suggesting that these could have been the first specimens of this species obtained by Dunker. The figures of *A.* (*M.*) *martensii* from a later publication (Dunker, 1882: pl. 10, figs. 7, 8) were reproduced in color by Shirai (1994: 28).

The shell from ZMB 108.685 lot perfectly matches the original description and illustration in Dunker (1879: pl. 23, fig. 2) in size, shape, color, and several unique features: (1) the outline of the abraded outer shell layer at the umbonal region and the submarginal area slightly posterior to the middle of the hinge line in RV; (2) the pattern or radial, commarginal, and irregular dark brown coloration on RV exterior; (3) the presence of the distinct thick commarginal growth line separating RV in the middle into distal and proximal halves (this feature is somewhat exaggerated in the figure); and (4) the concave outline of the ventral margin of anterior auricle in RV. The shell is depicted disarticulated in a staggered fashion with LV shifted upward. Part of the ligament in LV and the posteroventral margin of RV are damaged in the actual specimen. The same shell was later redrawn by Dunker (1882: pl. 10, fig. 7). The original illustration shows LV overlapped by RV, the former shifted slightly upward, so that the hinge area can be observed. In the figure made for a later publication, the shell is portrayed slightly differently, showing LV shifted diagonally, so that a larger part of the interior surface of LV is revealed. This later illustration also distinctly shows the damage of the ligament in two places in the resilifer in LV, the broken scales at the posteroventral margin of RV, and more extensively abraded outer shell layer in the posterodorsal region along the hinge line. Both of these features were not present in the original illustration despite similar level of detail, suggesting that the shell was indeed re-drawn (rather than the illustration copied) for the later publication. Two labels accompany ZMB 108.685:

Label 1: “*Avicula martensi* Dkr / Original. / Japan / Original zu Dunk Avicula / Taf 23 Fig 2 !” [handwritten by Martens except line 2 handwritten by Dunker];

Label 2: “Japan” [handwritten by Dunker].

ZMB 108.684a perfectly matches the figure by Dunker (1879: pl. 23, fig. 3) in size, shape, color, and several distinct features: (1) the short, dorsoventrally extended anterior auricle of RV; (2) the pattern of irregularly distributed dark brown blotches on RV exterior surface; (3) the commarginal dark brown stripe in the middle of RV; (4) the transverse light brown striations on the surface of flat overlapping scales distal to the dark brown commarginal stripe; and (5) the outline of a slightly damaged posteroventral margin of RV. The shell is depicted disarticulated in a staggered fashion with LV shifted upward. The second specimen in the same lot, ZMB 108.684b, is a disarticulated shell and is similar to the other specimen. Currently, both specimens are contained in the same container with by two labels, one containing the name “Lischke” and the other containing the name “Löbbecke.” Both names were written by Dunker. If Martens’ additional annotations on these labels are correct, the



FIGS. 44–55. Representative type specimens of *Avicula* from the Dunker collection. Shells arranged with hinge axis oriented horizontally. One of the syntypes is illustrated. Size is expressed as shell length. FIGS. 44–46: *A.* (*Meleagrina*) *martensii* Dunker, 1880; FIG. 44: Dunker's 1879: pl. 23, fig. 3) original illustration; FIG. 45: Syntype, exterior of RV (syntype; ZMB 108.684a, 43.8 mm); FIG. 46: Same specimen as Fig. 45, interior of LV. FIGS. 47–49: *A.* (*M.*) *olivacea* Dunker, 1872; FIG. 47: Dunker's (1872: pl. 4, fig. 5) original illustration; FIG. 48: Syntype, exterior of RV (ZMB 101.672, 55.8 mm); FIG. 49: Same specimen as Fig. 48, interior of LV. FIGS. 50–52: *A.* (*M.*) *pallida* Dunker, 1872; FIG. 50: Dunker's (1872: pl. 15, fig. 4) original illustration; FIG. 51: Syntype, exterior of RV (ZMB 112.821, 74.0 mm); FIG. 52: Same specimen as Fig. 51, interior of LV. FIGS. 53–55: *A. plicatula* Dunker, 1852; FIG. 53: Dunker's (1879: pl. 19, fig. 4) original illustration; FIG. 54: Holotype, exterior of RV (LMD/LÖB 124294a, 26.2 mm); FIG. 55: Same specimen as Fig. 54, interior of LV.

ZMB 108.684a specimen derives from the Löbbecke collection, and the ZMB 108.684b specimen—from the Lischke collection.

Label 1: "*Avicula Martensi* / Dkr / Japan. / Lischke. / Jap. Meeresconch. T p. 161 III, 112" [handwritten in brown ink by Dunker; words "*martensi*," "Drk," and line 5 in black ink added by Martens at a later date];

Label 2: "*Avicula* / *Martensi* Dkr / Japan / Löbbecke. / *Japonica* Dunk Avic 23, 3 !" [lines 1, 3, and 4 handwritten in brown ink by Dunker; lines 2 and 5 in black added by Martens at a later date].

The disarticulated shell from ZMB 108.683 perfectly matches the description and the illustration of RV by Dunker (1882: pl. 10, fig. 8) in size, shape, color and several unique features: (1) uniquely rounded (as opposed to typical triangulate) outline of the anterior auricle; (2) the number, pattern, and relative width of radial dark colored rays; and (3) the irregular damaged outline of the ventral margin. Four labels accompany ZMB 108.683:

Label 1: "Coll. Dunker" [printed];

Label 2: "*Avicula Martensii* / Dkr ⁜ / Japan / Dunk. ind moll / Jap Taf 10 Fig 8 ! /Dunker ind Moll./ Jap Tf 10 fig 8! / Original." [handwritten by Dunker; lines 4, 5, and the "⁜" symbol in line 2 added by Martens at a later date];

Label 3: "Original" [handwritten by Dunker];

Label 4: "Japan" [handwritten by Dunker].

Avicula nigrofusca Dunker, 1872 (Fig. 75)

Dunker, 1872: 20, pl. 5, fig. 4.

Type locality: Unknown.

Type material: Not located.

Comments: According to Dunker (1872: 20), *A. nigrofusca* possibly represents a young individual of *Avicula atlantica* Lamarck, 1819.

Avicula (*Meleagrina*) *olivacea* Dunker, 1872 (Figs. 47–49)

Dunker, 1872: 17, pl. 4, fig. 5.

Type locality: "Antillarum mare, mercatore teste qui concham venalem habuit" (Dunker, 1872: 17).

Syntype: ZMB 101.672, one pair of disarticulated valves; dimensions (RV): hinge length 52.1 mm, height 46.0 mm, length 55.8 mm.

Comments: The original description does not contain an explicit statement that the species was based on a single specimen. The shell from ZMB 101.672 collection perfectly matches the original description and the illustration in size, shape, and color. A distinctive semicircular notch at the anterior margin of the anterior auricle in RV and the pattern of well-preserved scales at the posterior margin unambiguously establish the identity of the shell. The shell is depicted articulated. Two labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed];

Label 2: "*olivacea* / Dunk ⁜ / Antillen / Dunk Avic. / Taf 4. Fig 5!" [lines 1 and 3 handwritten in pencil by Dunker; lines 2, 4, 5 in ink added by Martens at a later date].

Avicula (*Meleagrina*) *pallida* Dunker, 1872 (Figs. 50–52)

Dunker, 1872: 46, 47, pl. 15, fig. 4.

Type locality: Unknown.

Syntype: ZMB 112.821, one pair of disarticulated valves; dimensions (RV): hinge length 54.1 mm, height 82.0 mm, length 74.0 mm.

Comments: The original description does not contain an explicit statement that the species was based on a single specimen. It is significant that Dunker did not refer to *Avicula pallida* Conrad, 1837: 246, a senior primary homonym of *A.* (*M.*) *pallida* Dunker, 1872, whereas elsewhere he cited *Avicula nebulosa* Conrad, 1837: 246, another species from the same publication (and even from the same page). Evidently, Dunker was not familiar with Conrad's publication and relied on Reeve's (1857) interpretation of Conrad's species. Reeve (1857) omitted *A. pallida* in his revision and, consequently, so did Dunker. Dunker's *A.* (*M.*) *pallida* Dunker, 1872, a junior primary homonym of *Avicula pallida* Conrad, 1837, had not been renamed by later authors.

The shell from the ZMB collection perfectly matches the original description and the illustration in size, shape, color and several unique features: (1) conspicuous purple spots just posterior to umbo and in the middle of the exterior surface in RV; (2) short radial yellow streaks radiating from the umbo; and (3) the presence of scales only in posteroventral and anteroventral regions of RV. The shell is depicted articulated. The dimensions provided for the figured shell by Dunker (1872: 47; "quod exstat maximum a rostro usque ad alam extremam 92 m. m. longum") exceeds those obtained in the present study, most likely due to the damage along shell margins. Two labels accompany the lot:

Label 1: "*Avicula pallida* Dkr ⁜ / Dunk. Avic. Taf 15 fig 4! / - ?" [handwritten by Martens];

Label 2: "⁜ / pallida / Dkr." [handwritten by Martens].

Avicula peasei Dunker, 1872 (Figs. 92–94)

Avicula radiata Pease, 1862: 244.
Dunker, 1872: 24, 25, pl. 8, fig. 1.

Type locality: "*Hab*. Kingsmill Islands" (Pease, 1862: 244).

Syntypes (of *A. radiata* Pease, 1862, a replaced name): ZMB 112.902, five individual LV (posterior margins of two specimen damaged): hinge length 31.0–82.4 mm, height 19.9–48.7 mm, length 23.1–66.1 mm; one pair of disarticulated vales (RV): hinge length 45.5 mm, height 14.3 mm, length 17.3 mm; ZMB 20206, one LV (posterior auricle damaged): hinge length 41.7 mm, height 22.9 mm, length 25.2 mm.

Comments: Avicula peasei is a substitute name for *A. radiata* Pease, 1862, a junior primary homonym of *A. radiata* Leach, 1814. No shells unambiguously matching the specimen depicted by Dunker was found in the ZMB collection. The original description by Pease (1862) was not illustrated. Pease's type specimen (MCZ 298466) is similar to the shell illustrated by Dunker, but is articulated, unlike the figured specimen. Therefore, the source of Dunker's figure is unknown. Two ZMB lots contain specimens from the type locality sent to Dunker by Pease (Dunker, 1872: 24, "Plura specimina clar. Harper Pease benevole mecum communicavit"). Presumably, these specimens constituted part of the original type series of *Avicula radiata* Pease, 1862. (Pease's original description was not explicit with regard to the number of specimens upon which the species was based.) Two labels accompany ZMB 112.902:

Label 1: "Avicula radiata / (Pse) / Kingsmill Isles. / I have only rec^{d}. half / valves & these I send / you are the best [handwritten in ink by Pease] / 112.902" [handwritten in pencil by Köhler];

Label 2: "passt nicht zur ["does not match to"; here translated] / Abbildung 8,1 [handwritten in ink by Martens] / 112.902 [handwritten in pencil by Köhler]."

One label accompanies ZMB 20206:

Label: "Avicula Peasei / radiata Pease / Kingsmill /Pease / [unreadable] / 20206." [handwritten by Martens].

Avicula (*Meleagrina*) *petersii* Dunker, 1852 (Figs. 68, 69)

Dunker, 1852: 77, 78; 1880: 79.

Type locality: "Querimba. Attulit cl. Peters" (Dunker, 1852: 78).

Syntypes: ZMB 16.385a, one pair of disarticulated valves, posteroventral margin of RV slightly damaged; dimensions (RV): hinge length 42.9 mm, height 62.0 mm, length 68.5 mm; ZMB 16.385b, one pair of disarticulated valves, anterior margin of RV damaged and has a crack extending to the center of the valve; dimensions (RV): hinge length 42.9 mm, height 35.2 mm, length 36.5 mm RV; ZMB 975, soft tissues of two specimens in ethanol (corresponding to the dry shells from the ZMB 16.385a,b) and one juvenile pair of disarticulated valves.

Comments: Dunker indicated that the specimens upon which the description was based were collected by Peters and deposited at the ZMB collection (Dunker, 1852: 78). Peters conducted extensive collecting in Mozambique (Tete, Querimba, etc.) and much of his material is presently housed at ZMB. Dunker (1880: 79) stated that *A.* (*M.*) *petersii* is a variety of *A.* (*M.*) *lichtensteinii* Dunker, 1852. Unfortunately, he did not illustrate his specimens. The range of sizes provided by Dunker (1852: 78) implies that the species was based on multiple specimens. ZMB 16.385, catalogued by Martens as "*A. petersii*" in 1868, contains two shells collected by Peters from the type locality. The specimens from ZMB 16.385 and ZMB 975 (containing the corresponding soft tissues) are considered here as syntypes. Four labels accompany ZMB 16.385:

Label 1 (recto): "Meleagrina / Petersii/ Dkr. ⁜ / Z. Mal 1852 p. 77 / occa Rv 24 / Thier in Weingeist ["animal in alcohol"; here translated; referring to lot ZMB 975] / Querimba. / Peters / 16.385" [lines 1–3, 6–8 handwritten probably by Troschel, lines 4, 5 handwritten by Martens];

Label 1 (verso): "~~Avicula~~ / ~~brevicauda~~, / ~~Desh~~. / ~~Their in Weingeist~~. / ~~Querimba~~. / ~~Peters~~." [old typical museum label, handwritten in ink, probably by Troschel; crossing-out in pencil by unidentified person];

Label 2: "Petersi Dkr ⁜ / Mal. Zeitschr 1852. / flabellum / Rv." [handwritten by Martens];

Label 3: "bicolor / Dkr / ob die Phazies dazu gehört? / sie ähnelt der / maxima ["bicolor / Dkr / if this facies belongs to it? / it is similar to / maxima"; here translated]" [handwritten by Martens];

Label 4: "Zoologisches Museum Berlin. [printed] / Avicula 16385 / ⁜ petersi Dkr. / Querimba Ins. Peters" [lines 2, 3 handwritten by Köhler, line 4 handwritten by unidentified person; standard ZMB label].

One label accompanies ZMB 975:

Label: "Avicula / pertersi Dunker / 975 / Querimba Ins. Peters" [handwritten by Martens].

Avicula plicatula Dunker, 1852 (Figs. 53–55)

Dunker, 1852: 75, 76; 1872: 56; 1879: 57, pl. 19, fig. 4.

Type locality: Unknown.

Holotype (fixed by monotypy): LMD/LÖB 124294a, one pair of disarticulated valves (extremities of posterior auricles of both valves broken off); dimensions (RV): hinge length 31.5 mm, height 20.2 mm, length 26.2 mm.

Comments: The revised description stated that the species was based on a single specimen from the Scheepmaker collection ("Specimen hic depictum originale ad collectionem calr. Scheepmaker…") that later became incorporated into the Löbbecke collection (Dunker, 1872: 56). This is confirmed by the words "Original" and "Unicum" on the labels written by Dunker and Löbbecke. The shell from the LMD collection perfectly matches the original description and the illustration of the articulated shell in shape, size, and color, and two particular features: the jagged outlines of the ventral margin of the posterior auricle and the anterior margin, and the pattern of the abrasion in the umbonal region in RV. Four labels accompany the LMD lot:

Label 1: "Scheepm. 1121 / Avicula / plicatula Dunker / Original / Unicum / Dunker Mart. Chem. / Tab. 19 f. 4" [handwritten by Löbbecke];

Label 2: "plicatula Dkr. / unicum." [handwritten by Dunker];

Label 3: "Avicula plicatula Dkr. / Unicum / Scheepm / 1 [handwriting unidentified] Löbbecke-Museum" [old printed label of the Löbbecke Museum, c. 1910];

Label 4: "Avicula plicatula DUNKER / 1 Ex. / Unicum / Scheepmaker / Vorlagez. d. Abb. i. M. u. Ch. / Bd.VII, 3, Tafel 19, Figur 4. [typed] / Löbbecke-Museum" [printed].

Avicula (*Meleagrina*) *prasina* Dunker, 1872 (Figs. 56–58)

Dunker, 1872: 53, pl. 18, fig. 4.

Type locality: Unknown.

Syntype: ZMB 112.808, one pair of disarticulated valves; dimensions (RV): hinge length 31.2 mm, height 37.1 mm, length 35.5 mm.

Comments: The original description does not contain an explicit statement that the species was based on a single specimen. The dimensions provided for the figured shell ("Testa a cauda extrema usque ad alae anticam partem 46 m. m. longa"; Dunker, 1872: 53) only slightly exceed those obtained in the present study. This discrepancy is most likely due to the slight damage the shell margins and the tip of the posterior auricle. The shell from the ZMB collection perfectly matches the original description and the illustration in shape, unusually dark green color, and the following particular features: (1) several commarginal light green stripes in the umbonal area; (2) the presence of flat, short, wide scales on most of RV surface; and (3) and the lighter colored (yellow-brown) anterior auricle in RV. Three labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed label; glued to inner surface of LV];

Label 2: "N. Austr. / *flexuosa* Rv" [handwriting unidentified];

Label 3: "Zoolog. Museum Berlin" [printed].

Avicula (*Meleagrina*) *reentsii* Dunker, 1872 (Fig. 76)

Dunker, 1872: 9, 10, pl. 2, figs. 1, 2.

Type locality: "Habitat in Mari rubro" (Dunker, 1872: 10).

Type material: Not located.

Comments: The depicted shell originates from the Reents collection in Hamburg (Dunker, 1872: 10).

Avicula (*Meleagrina*) *reeveana* Dunker, 1872

Avicula fimbriata Reeve, 1857: Species 25, pl. 9, fig. 25.

Type locality: "North-west coast of Australia (under pieces of coral at low water)" (Reeve, 1857).

Comments: A. reeveana is a substitute name for *A. fimbriata* Reeve, 1857, a junior primary homonym of *Avicula* (*Meleagrina*) *fimbriata* Dunker, 1852. The illustration was based on the specimen from the Cuming collection figured by Reeve (1857: pl. 9, fig. 25).

Avicula rufa Dunker, 1848 (Figs. 95–97)

Dunker, 1848: 180; 1879: 58, pl. 19, figs. 7, 8.

Type locality: "Insula Java" (Dunker, 1848: 180).

Syntype: ZMB 112.966, one pair of disarticulated valves; dimensions (RV): hinge length 66.4 mm, height 36.4 mm, length 39.3 mm. Probable *syntypes:* ZMB 16.353, two pairs of disarticulated valves; dimensions (RV): hinge length 53.5 mm, height 19.1 mm, length 28.0

mm; hinge length 42.6 mm, height 11.4 mm, length 18.2 mm (posterior auricle damaged).

Comments: The types were stated to originate from Java and were communicated to Dunker by Philippi (Dunker, 1848: 180). This suggests that some of the specimens upon which the species was based might be in the Philippi collection, possibly still extant at MNHS. None of the specimens at ZMB perfectly match the shells depicted by Dunker (1879: pl. 19, figs. 7, 8). ZMB 112.966 contains a shell from the Dunker collection that is very similar to the figured specimens. This specimen was obtained by Winter from Java and was most likely part of the original type series and is here considered a syntype.

Four labels accompany ZMB 112.966:

Label 1: "*Avicula rufa*, / Dkr. / Java. / Winter." [handwritten by Dunker; original label of the Dunker collection];

Label 2: "*Avicula*. / Flores. K." [handwritten by Dunker; original label of the Dunker collection];

Label 3: "coll. Dunker" [printed; glued to verso of Label 1];

Label 4: "Zoologisches Museum Berlin. [printed] / Java Dunker" [handwriting unidentified; standard ZMB label].

The two shells in ZMB 16.353 were cataloged as types of *A. rufa* in 1868 (catalog entry handwritten by Martens). These shells are similar but not identical to either of the two articulated shells illustrated by Dunker (1879: pl. 19, figs. 7, 8). The larger shell of the two pairs of disarticulated valves lot is similar to the depicted shells in overall shape and color but is different in (1) the extent of the posterior auricle; (2) the shape of the anterior auricles as observed in RV; (3) the abraded outer shell layer at the umbo; and (4) some marginal fractures. (The damage, however, could have occurred subsequently to the illustration.)

One label accompanies ZMB 16.353:

Label: "*Avicula* / *rufa*, / Dunker / Mke et Pfr. Zeitschr. f. / Malacoz. 1848 p. 180 / cf inquinata Rv / 16353" [handwriting unidentified; lines 6 and 7 handwritten by Martens; standard ZMB label].

Avicula (*Meleagrina*) *scheepmakeri* Dunker, 1872 (Figs. 59–61)

Dunker, 1872: 6, pl. 1, fig. 1.

Holotype (fixed by monotypy): ZMB 112.911, one pair of disarticulated valves; dimensions: hinge length 54.6 mm, height 87.3 mm, length 93.6 mm.

Type locality: Unknown.

Comments: Dunker's mention of the single original specimen upon which the species was based ("Das Original"; Dunker, 1872: 6) and the presence of Dunker's labels marked as "Original" warrant the recognition of the specimen from the ZMB collection as a holotype by monotypy. The shell derived from the Scheepmaker collection (Dunker, 1872: 6) that later became incorporated into the collection of Wilhelm Arnold in Nordhausen (Dunker, 1880: 79), which is also currently housed at ZMB. According to Dunker (1880: 79), the specimen came from Solomon Islands, as communicated by Dr. Augustus Sutor who, in turn, was informed by Dr. Krefft from Sydney. The black-and-white photographs of the holotype were published by Ranson (1961: pl. 14, fig. 2 [LV exterior], fig. 5 [RV exterior]; pl. 15, fig. 2 [LV interior], fig. 5 [RV interior]).

The specimen from ZMB 112.911 matches the original description and the illustration in size, shape, color, and several unique features: (1) the outline of the abraded outer shell layer near the umbo and close to the byssal notch in RV; (2) the outline of the damaged area of RV ventral margin; (3) the radial fracture extending from the ventral margin of RV to about one fourth of shell height; (4) the indentation on the hinge line posterior to umbones; (5) the outline of shell margin in the ventral part of LV opposing the byssal notch; and (6) the color pattern of two wide dark stripes on the inner surface of the anterior margin or LV. The shell is depicted with valves diagonally staggered. Two labels accompany the ZMB lot:

Label 1: "*Avicula scheepmakeri* / Dkr. / Mus. Scheepmaker / Original zu meiner ["My original"; here translated] / Abbildung! Dkr." [handwritten by Dunker];

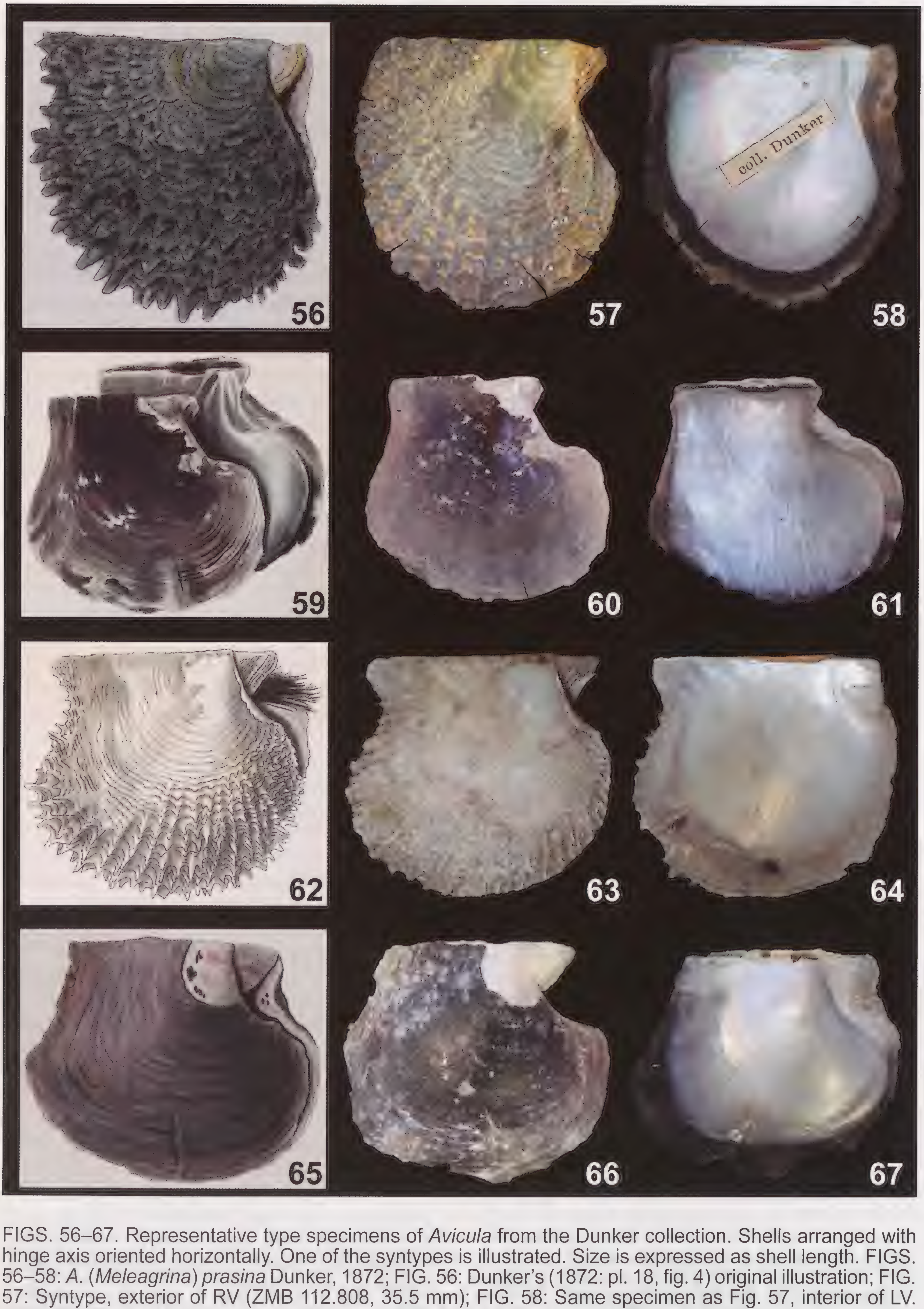
Label 2: "*Meleagrina scheepmakeri* / Dkr. / Original zu Dunkers Abbild. / M.Ch. fol.6 taf 1 fig.1" [handwriting unidentified; label of the Arnold collection].

Avicula serrulata Dunker, 1848 (Figs. 98–100)

Dunker, 1848: 178, 179; 1872: 18, 19, pl. 5, figs. 1, 2.

Type locality: "Habitat ad insulas Moluccanas" (Dunker, 1848: 179).

Syntype: ZMB 108.695, one pair of disarticulated valves; dimensions (RV): hinge length 92.5 mm, 61.2 height mm, length 81.9 mm.



FIGS. 56–67. Representative type specimens of *Avicula* from the Dunker collection. Shells arranged with hinge axis oriented horizontally. One of the syntypes is illustrated. Size is expressed as shell length. FIGS. 56–58: *A.* (*Meleagrina*) *prasina* Dunker, 1872; FIG. 56: Dunker's (1872: pl. 18, fig. 4) original illustration; FIG. 57: Syntype, exterior of RV (ZMB 112.808, 35.5 mm); FIG. 58: Same specimen as Fig. 57, interior of LV. FIGS. 59–61: *A.* (*M.*) *scheepmakeri* Dunker, 1872; FIG. 59: Dunker's (1872: pl. 1, fig. 1) original illustration; FIG. 60: Holotype, exterior of RV (ZMB 112.911, 93.6 mm); FIG. 61: Same specimen as Fig. 60, interior of LV. FIGS. 62–64: *A.* (*M.*) *tamsiana* Dunker, 1852; FIG. 62: Dunker's (1872: pl. 4, fig. 1) original illustration; FIG. 63: Syntype, exterior of RV (ZMB 101.675, 60.1 mm); FIG. 64: Same specimen as Fig. 63, interior of LV. FIGS. 65–67: *A.* (*M.*) *tristis* Dunker, 1872; FIG. 65: Dunker's (1872: pl. 14, fig. 3) original illustration; FIG. 66: Syntype, exterior of RV (ZMB 108.680, 57.0 mm); FIG. 67: Same specimen as Fig. 66, interior of LV.

Comments: The species was based on multiple specimens. The shell from the ZMB collection perfectly matches the original description and the illustration by Dunker (1872: pl. 5, fig. 1) in size, shape, color, and several unique features: (1) the protruding and distally widening anterior auricle; (2) the radial fracture extending from the midventral margin to approximately half-height of the shell; (3) the outline of the fractures at the dorsal margin; and (4) the outline of the abraded outer shell layer at the umbonal region. The specimen was communicated by Winter (Dunker, 1848: 179). The second figure (Dunker, 1872: pl. 5, fig. 2) was copied from the illustration by Reeve (1857: pl. 4, fig. 5). Reeve referred to this species as *Avicula lata* Gray, and based his illustration on a specimen from the Cuming collection from "Port Essington and Raines' Island, Torres Straits, Australia" (Reeve, 1857: Species 5). Four labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed];

Label 2: "*Avicula serru- / lata*. Dkr. / Insulae Molluc. / com. et. Winter" [handwritten by Dunker; original label of the Dunker collection];

Label 3: "*Avicula serrulata*. / Dkr. ⁜ / Monog. Tf. 5 fig 1.2 / Molukken / Winter. / Winter" [handwritten by Dunker; lines 3, 4, 6, and the "⁜" symbol in line 2 added by Martens at a later date; original label of the Dunker collection];

Label 4: "*serrulata* / Dkr ⁜" [handwritten by Martens].

Avicula spadicea Dunker, 1852 (Figs. 77, 78)

Dunker, 1852: 73; 1872: 31, pl. 10, figs. 1, 8.

Type locality: "Habitat in Mari Rubro; attulit. Cl. Rob. Rodatz" (Dunker, 1852: 73).

Type material: Not located.

Comments: According to Dunker (1852: 73; 1872: 31), the specimens upon which the original description was made came from the Rodatz collection. The location of the type material is unknown.

Avicula spadicea var. Dunker, 1879

Avicula rutila Reeve, 1857: Species 19, pl. 8, fig. 19; Dunker, 1879: 62, pl. 22, fig. 1.

Type locality: "Australia" (Reeve, 1857).

Comments: Dunker (1879: 62) considered *Avicula rutila* Reeve, 1857, to be a variety of *A. spadicea* Dunker, 1852. The illustration was based on the specimen from the Cuming collection figured by Reeve (1857: pl. 8, fig. 19).

Avicula straminea Dunker, 1852
(Figs. 101–103)

Dunker, 1852: 74, 75; 1872: 26, 27, pl. 9, fig. 1.

Type locality: Unknown.

Syntype: ZMB 112.865, one pair of articulated valves; dimensions (RV): hinge length 81.6 mm, height 36.3 mm, length 54.3 mm.

Comments: The original description does not contain an explicit statement that the species was based on a single specimen. In the subsequent revision, Dunker (1872: 26) mentioned the presence of other specimens, probable syntypes, in his collection, although it could not be established if these specimens were available to Dunker at the time of the original description. The specimens from ZMB 112.865 closely matches the original description and illustration size, shape, color, and several distinctive features: (1) the pattern of two folds on the exterior surface of the anterior auricle of RV; (2) the outlines of the slightly damaged ventral margin of RV and of the exposed anterior margin of LV; (3) the outline of the irregular fracture of the outer layer of RV; and (4) a distinct curved line (dark brown in the drawing but much lighter in the actual specimen) in the middle of the depressed area demarcating the posterior auricle from the posterior margin of RV. The damaged tip of the posterior auricle of RV and the barnacle incrustation of the umbonal region are not illustrated. The shell is depicted articulated. The specimen originates from the Gruner collection as was stated in the original description (Dunker, 1852: 74; 1872: 26), and, according to the labels, must have subsequently been incorporated into the Paetel collection. The original description does not indicate type locality ("patria ignota"), but in his revision, Dunker (1872: 26) noted that the species if found in the eastern Indian Ocean. Three labels accompany the ZMB lot:

Label 1: "coll. Paetel" [printed];

Label 2: "*Avicula* / ~~*fimbriata* Dkr.~~ / 1. Austol. / 300m" [handwritten in ink by Paetel; the second line crossed out in pencil by unidentified person; original label of the Paetel collection];

Label 3: "*straminea* / Dkr." [handwritten by Martens].

Avicula (*Meleagrina*) *tamsiana* Dunker, 1852
(Figs. 62–64)

Dunker, 1852: 78; 1872: 15, 16, pl. 4, figs. 1, 2 (var.).

Type locality: "Prope Puerto Cabello legit cl. G. Tams, Med. Dr. (La Guayra teste Koch)" (Dunker, 1852: 78).

Syntype: ZMB 101.675, one pair of disarticulated valves; dimensions (RV): hinge length 52.5 mm, height 54.5 mm, length 60.1 mm.

Comments: The original description does not contain an explicit statement that the species was based on a single specimen. The specimen from ZMB 101.675 matches the original description and the illustration in size, shape, color, and two unique features: the outward folding of the anterodorsal margin of RV close to the byssal notch, and the pattern of dark blotches on the dorsal and posterior side of the umbo. The fact that the shell is depicted articulated with the byssus extending through the byssal notch suggests that valves were separated at a later date and the byssus was lost. Dunker obtained his specimen(s) from Tams (Dunker, 1852: 78; 1872: 16). Three labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed];

Label 2: "*Avicula* (*Melea / grina*) *Tamsii, / Tamsiana* / Dkr. ⁜ / Pto Cabello / Tams / Avic. 4 fig. 1 Tam5." [handwritten by Dunker; lines 3, 6, and the "⁜" symbol in line 4 added by Martens at a later date; original label of the Dunker collection];

Label 3: "Zoologisches Museum Berlin. [printed] / Puerto Cabello Dunker" [handwriting unidentified; standard ZMB label].

Avicula tortirostris Dunker, 1848
(Figs. 104–106)

Dunker, 1848: 179, 180; 1872: 37, 38, pl. 11, fig. 6.

Type locality: "Insula Java" (Dunker, 1848: 180).

Syntype: ZMB 112.866, one pair of disarticulated valves; dimensions (RV): hinge length 84.2 mm, height 23.5 mm, length 31.6 mm.

Comments: The original description does not contain an explicit statement that the species was based on a single specimen. The shell in ZMB 112.866 closely matches the original description and illustration in shape, color, and several unique features: (1) anteriorly extending anterior auricle in RV with atypical fold; (2) the outline of the fracture proximal to the dorsal margin of RV; and (3) the outline of the broken margin immediately ventral to the byssal notch in LV. The shell is shown articulated. The fact that the dimensions provided for the figured shell by Dunker (1872: 37, 38; "Long. marginis superioris subrecti ab umbonibus usque ad extremam caudam 65 m. m., long. rostri 21, long. ab umbonibus ad finem alae 50") exceed those obtained in the present study, is most likely due to the damage along the ventral shell margins and the posterior auricle. The tip of the posterior auricle in and the posteroventral margin in RV are more severely damaged than illustrated. Four labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed];

Label 2: "*Avicula tor. / tirostris* Dkr. / Ins. Java / Mad. Schnell" [handwritten by Dunker; original label of the Dunker collection];

Label 3: "*tortirostris* Rv" [handwritten by Martens];

Label 4: "Dunker / taf 11 fig 6" [handwritten by Martens].

Avicula (*Meleagrina*) *tristis* Dunker, 1872
(Figs. 65–67)

Dunker, 1872: 44, pl. 14, fig. 3.

Type locality: Unknown.

Syntype: ZMB 108.680, one pair of disarticulated valves; dimensions (RV): hinge length 43.3 mm, height 52.5 mm, length 57.0 mm.

Comments: The original description does not contain an explicit statement that the species was based on a single specimen. The shell from the ZMB collection perfectly matches the original description and the illustration in size, shape, and color. The shell is unambiguously defined by the following features of RV: (1) the radial fracture of the outer shell layer in the midventral part of the valve; (2) the broad and rounded outline of the abraded prismatic layer in the umbonal region; and (3) the fracture originating from the dorsal part of the umbonal abraded region that extends posteriorly along the hinge line. The margins of the specimen are more strongly damaged than depicted. The shell is depicted articulated. Two labels accompany the ZMB lot:

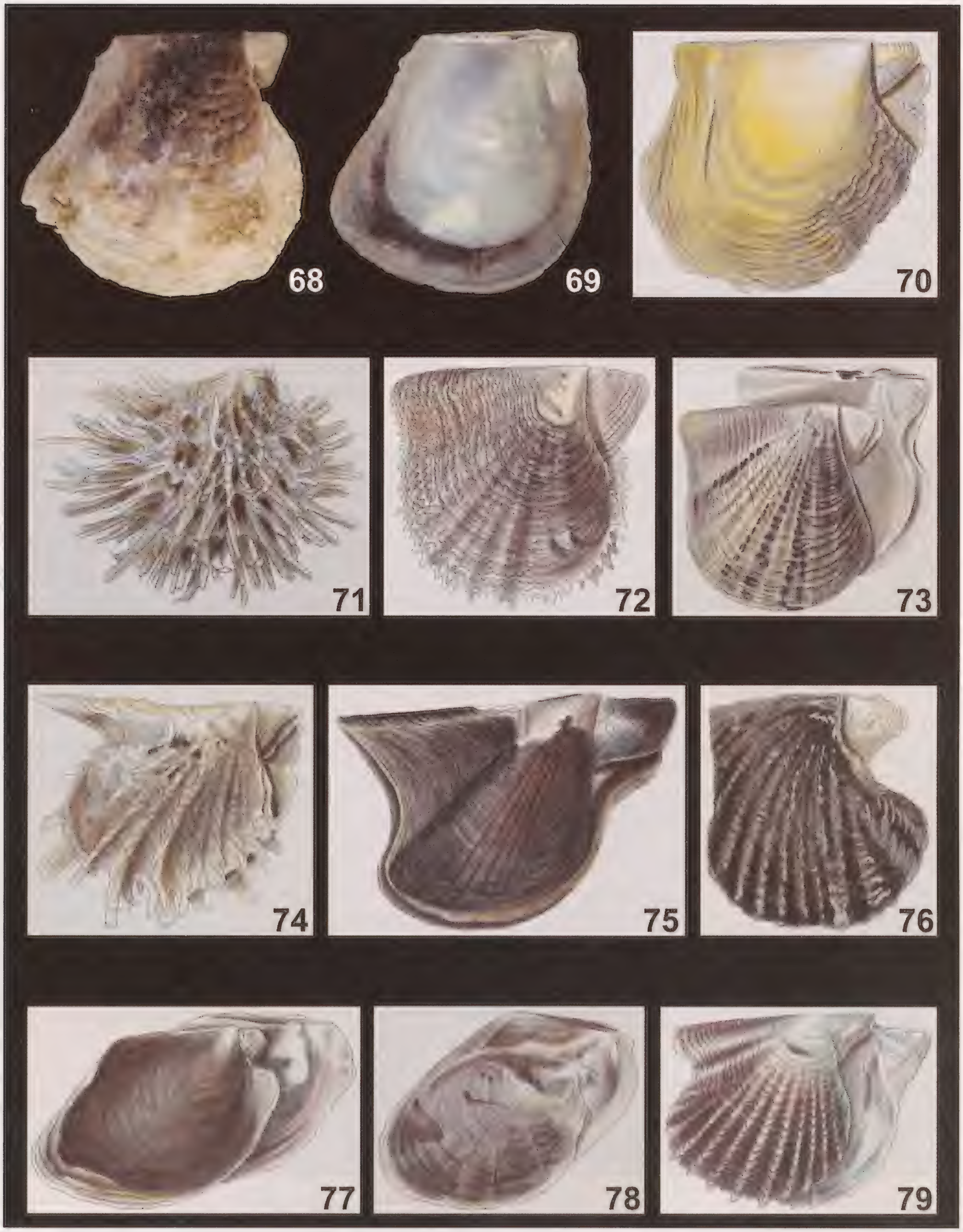
Label 1: "*Avicula nigra* / (Singapore A. Gould) Glot / =*tristris* Dkr. ⁜ / Avic. Monogr. Taf 14 / Fig 3" [handwritten by Martens];

Label 2: "*nigra* A Gould / *tristis* / ⁜ / Dkr" [handwritten by Martens].

Avicula undata Dunker, 1879
(Figs. 107–109)

Dunker, 1879: 68, pl. 24, fig. 2.

Type locality: Unknown.



FIGS. 68–79. Representative type specimens of *Avicula* from the Dunker collection. Shells arranged with hinge axis oriented horizontally. One of the syntypes is illustrated. Size is expressed as shell length. FIGS. 68, 69: *A.* (*Meleagrina*) *petersii* Dunker, 1852; FIG. 68: Syntype, exterior of RV (ZMB 16.385a, 68.5 mm); FIG. 69: Same specimen as Fig. 68, interior of LV. FIG. 70: *A.* (*M.*) *citrina* Dunker, 1852; Dunker's (1872: pl. 3, fig. 4) original illustration. FIG. 71: *A.* (*M.*) *echinus* Dunker, 1872; Dunker's (1872: pl. 13, fig. 2.) original illustration. FIGS. 72, 73: *A.* (*M.*) *fimbriata* Dunker, 1852; Dunker's (1872: pl. 3, figs. 2, 6) original illustrations. FIG. 74: *A.* (*M.*) *grisea* Dunker, 1872; Dunker's (1872: pl. 2, fig. 5) original illustration. FIG. 75: *A. nigrofusca* Dunker, 1872; Dunker's (1872: pl. 5, fig. 4) original illustration. FIG. 76: *A.* (*M.*) *reentsii* Dunker, 1872; Dunker's (1872: pl. 2, fig. 1) original illustration. FIGS. 77, 78: *A. spadicea* Dunker, 1852; Dunker's (1872: pl. 10, figs. 1, 8) original illustrations. FIG. 79: *A.* (*M.*) *varia* Dunker, 1872; Dunker's (1872: pl. 4, fig. 6) original illustration.

Syntype: ZMB 108.688, one pair of disarticulated valves; dimensions (RV): hinge length 63.2 mm, height 19.9 mm, length 27.9 mm.

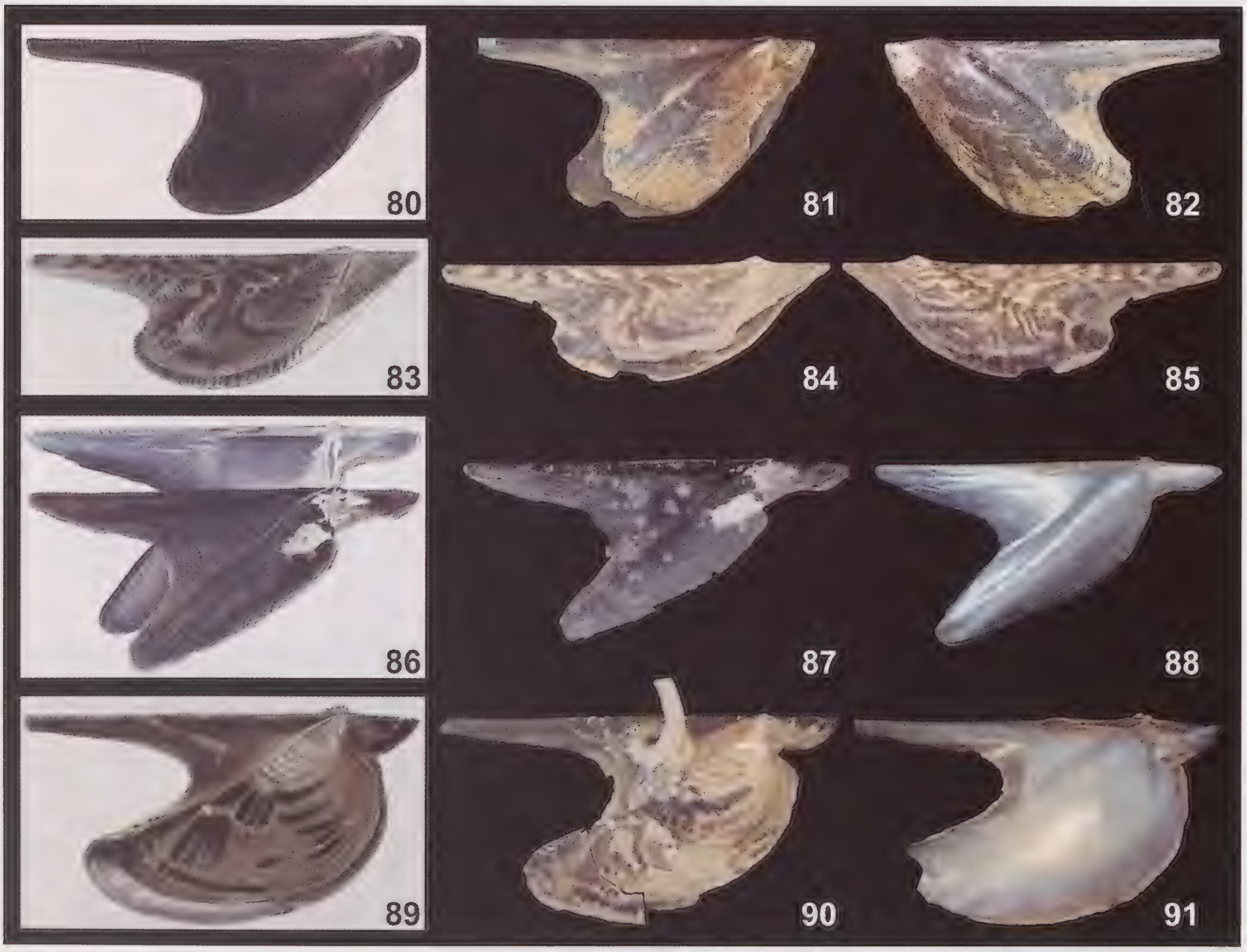
Comments: The original description does not contain an explicit statement that the species was based on a single specimen. The single shell present in the type lot closely matches the original description and illustration in size, shape, color, and several unique features: (1) a radial green stripe passing through the depression separating the posterior auricle from the posterodorsal margin of RV; (2) two folds radiating from the umbo on the exterior surface of the anterior auricle of RV; and (3) somewhat lighter coloration at the anterior half of RV surface. The shell is depicted articulated. Three labels accompany the ZMB lot:

Label 1: "coll. Dunker" [printed label];
Label 2: "Taf. 24. f. 2. / *A. undata* / Dkr" [handwritten by Dunker];
Label 3: "Landauer" [handwritten by Dunker];
Label 4: "*undata* Dkr. ⁜" [handwritten by Martens].

Avicula (*Meleagrina*) *varia* Dunker, 1872
(Fig. 79)

Dunker, 1872: 17, 18, pl. 4, fig. 6.

Type locality: "Hanc speciem colore variabilem beatus Albertus Rodatz e Mari rubo quondam attulit" (Dunker, 1872: 18).



FIGS. 80–91. Representative type specimens of *Avicula* from the Dunker collection. Shells arranged with hinge axis oriented horizontally. One of the syntypes is illustrated. Size is expressed as shell length. FIGS. 80–82: *A. atrata* Dunker, 1872; FIG. 80: Dunker's (1879, pl. 19, fig. 1) original illustration; FIG. 81: Probable syntype, exterior of RV (ZMB 114.467a, 48.7 mm); FIG. 82: Same specimen as Fig. 81, exterior of RV. FIGS. 83–85: *A. coturnix* Dunker, 1879; FIG. 83: Dunker's (1879: pl. 23, fig. 5) original illustration; FIG. 84: Syntype, exterior of RV (ZMB 108.689a, 22.2 mm); FIG. 85: Same specimen as Fig. 84, exterior of LV. FIGS. 86–88: *A. cypsellus* Dunker, 1872; FIG. 86: Dunker's (1872: pl. 8, fig. 4) original illustration; FIG. 87: Syntype, exterior of RV (LMD/LÖB 132969a, 51.7 mm); FIG. 88: Same specimen as Fig. 87, interior of LV. FIGS. 89–91: *A. jeffreysii* Dunker, 1880; FIG. 89: Dunker's (1879: pl. 24, fig. 3) original illustration; FIG. 90: Syntype, exterior of RV (ZMB 108.691, 39.5 mm); FIG. 91: Same specimen as Fig. 90, interior of LV.

Type material: Not located.

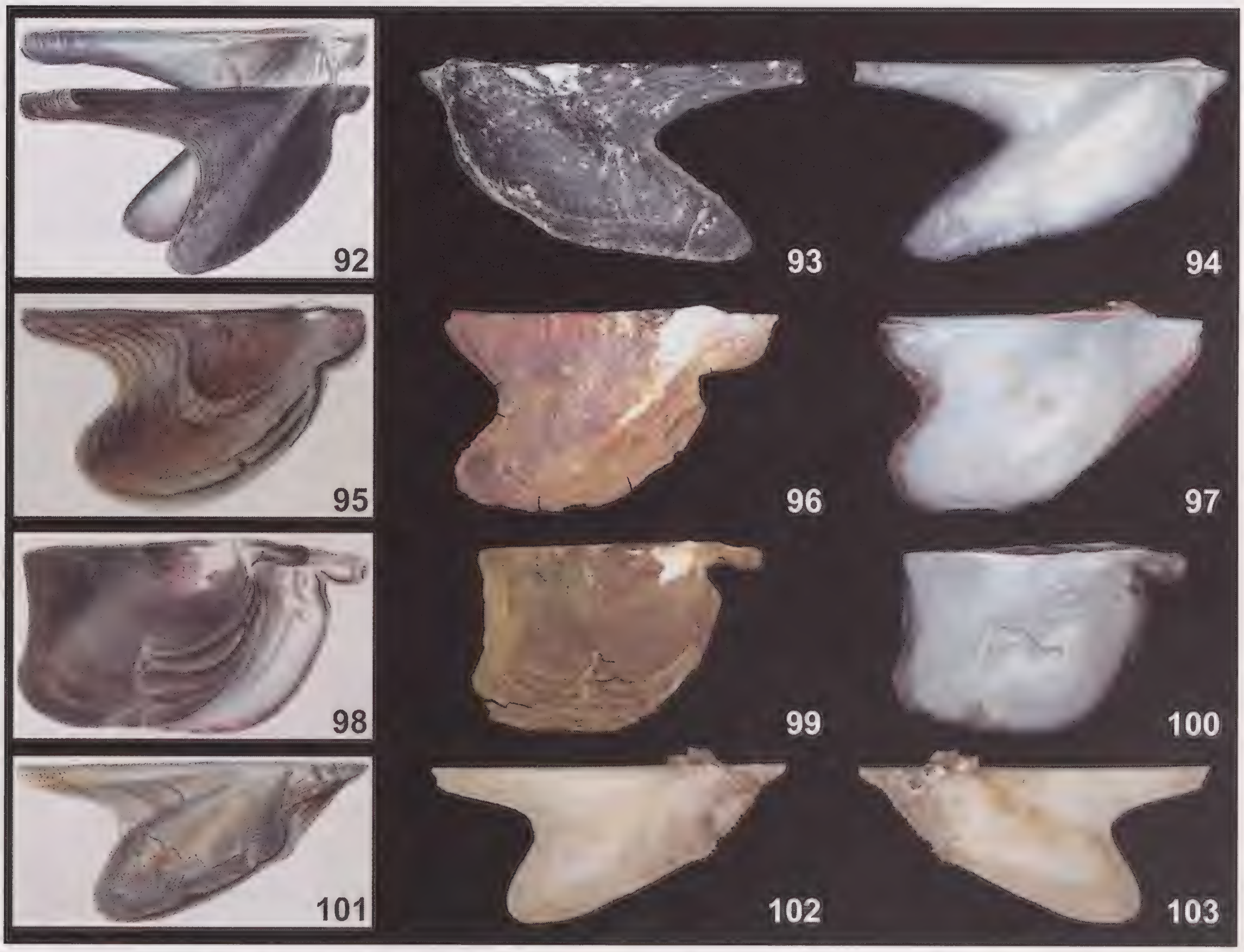
Comments: No shell from the Dunker collection at ZMB matches a rather unspecific figure of a disarticulated shell (LV shifted to right). The original description refers to Rodatz, who might have owned the specimen upon which the species was based. Martens identified ZMB 112.965 from the Dunker collection as *A.* cf. *varia* from the Red Sea. None of the specimens (three pairs of disarticulated valves and two single right valves), however, unquestionably match the illustration, and no original labels are preserved. Five labels accompany the ZMB lot:

Label 1: "cf A varia / Dunk Tf 4 Fig 6 / (Rothes Meer)" [handwritten by Martens];
Label 2: "coll. Dunker" [printed label, glued to verso of Label 1];
Label 3: "varia Dkr" [handwritten by Martens];
Label 4: "varia Dkr" [handwritten by Martens];
Label 5: "Zoologisches Museum Berlin. [printed] / Rotes Meer Dunker" [handwriting unidentified; standard ZMB label].

Avicula venezuelensis Dunker, 1872
(Fig. 115)

Dunker, 1872: 19, pl. 5, fig. 3.

Type locality: "Litus Venezuelense" (Dunker, 1872: 19).



FIGS. 92–103. Representative type specimens of *Avicula* from the Dunker collection. Shells arranged with hinge axis oriented horizontally. One of the syntypes is illustrated. Size is expressed as shell length. FIGS. 92–94: *Avicula peasei* Dunker, 1872; FIG. 92: Dunker's (1872: pl. 8, fig. 1) original illustration; FIG. 93: Syntype, exterior of LV (ZMB 112.902, 64.0 mm); FIG. 94: Same specimen as Fig. 93, interior of LV. FIGS. 95–97: *A. rufa* Dunker, 1848; FIG. 95: Dunker's (1879: pl. 19, fig. 8) original illustration; FIG. 96: Probable syntype, exterior of RV (ZMB 112.966, 39.3 mm); FIG. 97: Same specimen as Fig. 96, interior of LV. FIGS. 98–100: *A. serrulata* Dunker, 1848; FIG. 98: Dunker's (1872: pl. 5, fig. 1) original illustration; FIG. 99: Syntype, exterior of RV (ZMB 108.695, 81.9 mm); FIG. 100: Same specimen as Fig. 99, interior of LV. FIGS. 101–103: *A. straminea* Dunker, 1852; FIG. 101: Dunker's (1872: pl. 9, fig. 1) original illustration; FIG. 102: Syntype, exterior of RV (ZMB 112.865, 54.3 mm); FIG. 103: Same specimen as Fig. 102, exterior of LV.

Type material: Not located.

Comments: There is no information on the type material.

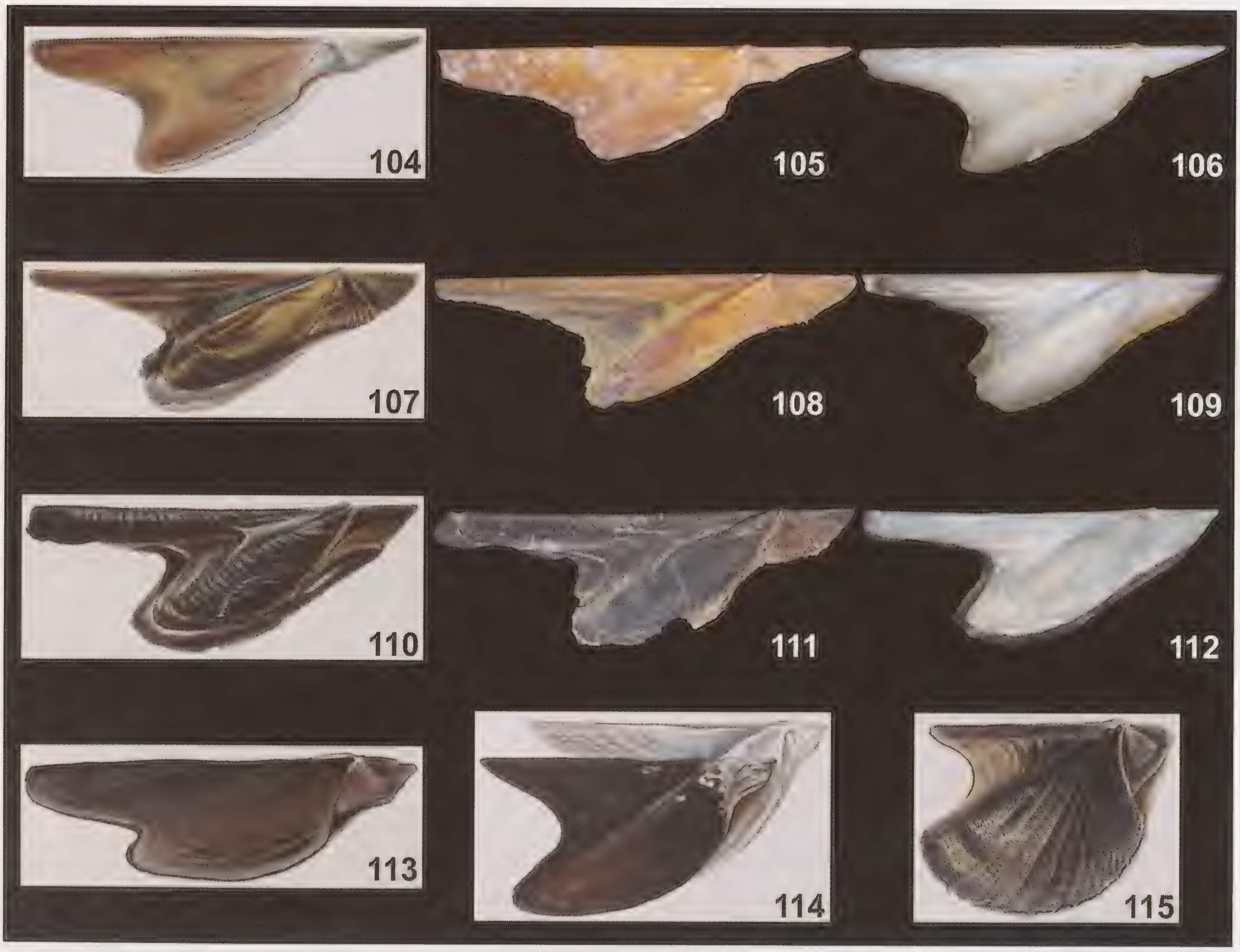
Avicula versicolor Dunker, 1879
(Figs. 110–112)

Dunker, 1879: 68, pl. 24, figs. 1, 4.

Type locality: Unknown.

Syntypes: ZMB 108.686, one pair of articulated valves; dimensions (both valves): hinge length 55.5 mm, height 21.1 mm, length 30.9 mm; ZMB 108.687, one pair of disarticulated valves; dimensions (RV): hinge length 62.5 mm, height 19.4 mm, length 28.5 mm.

Comments: The species was based on multiple specimens. Both specimens, most likely conspecific, closely match the original description and illustrations in size, shape, and several unique features. The syntype ZMB 108.686 is similar to the shell depicted in Dunker's fig. 1 (Dunker, 1879: pl. 24) in (1) the outline of extensively damaged shell margin in both valves and the tips of the posterior auricles; (2) the pattern of commarginal stripes in the posterodorsal region and the auricular sinus of RV; and (3) the general pattern of coloration (while the dark brown-greenish part of RV surface in the illustration does not perfectly match the bright green color in the



FIGS. 104–115. Representative type specimens of *Avicula* from the Dunker collection. Shells arranged with hinge axis oriented horizontally. One of the syntypes is illustrated. Size is expressed as shell length. FIGS. 104–106: *A. tortirostris* Dunker, 1848; FIG. 104: Dunker's (1872: pl. 11, fig. 6) original illustration; FIG. 105: Syntype, exterior of RV (ZMB 112.866, 31.6 mm); FIG. 106: Same specimen as Fig. 105, interior of LV. FIGS. 107–109: *A. undata* Dunker, 1879; FIG. 107: Dunker's (1879: pl. 24, fig. 2) original illustration; FIG. 108: Syntype, exterior of RV (ZMB 108.688, 27.9 mm); FIG. 109: Same specimen as Fig. 108, interior of LV. FIGS. 110–112: *A. versicolor* Dunker, 1879; FIG. 110: Dunker's (1879: pl. 24, fig. 4) original illustration; FIG. 111: Syntype, exterior of RV (ZMB 108.687, 28.5 mm); FIG. 112: Same specimen as Fig. 111, interior of LV; FIG. 113: *A. brevialata* Dunker, 1872; Dunker's (1879: pl. 19, fig. 3) original illustration. FIG. 114: *A. gruneri* Dunker, 1872; Dunker's (1872: pl. 18, fig. 1) original illustration; FIG. 115: *A. venezuelensis* Dunker, 1872; Dunker's (1872: pl. 5, fig. 3) original illustration.

actual specimen). The shell is depicted articulated. The other specimen, ZMB 108.687, corresponds to the second figure accompanying the original description (Dunker, 1879: pl. 24, fig. 4) in having (1) the outline of the damage ventral edge of the posterior auricle in RV; (2) the presence of two pale stripes in the depressed area separating the posterior auricle from the posterodorsal margin of RV; and (3) a single, whitish, short stripe running perpendicular to shell margin of RV. This shell is more severely damaged along margins than depicted. Although the specimen is shown articulated, the valves are presently separated. Two labels accompany ZMB 108.687:

Label 1: "coll. Dunker" [printed label];
Label 2: "Taf. 24. f. 4. / *A. versicolor* / Dkr" [handwritten by Dunker].

Three labels accompany ZMB 108.686:

Label 1: "coll. Dunker" [printed label];
Label 2: "*versicolor* / Dkr ⁜" [handwritten by Martens];
Label 3: "Taf. 24. / fig. 1. / *A. versicolor* Dkr" [handwritten by Dunker].

FIGURED NON-TYPE MATERIAL FROM THE DUNKER COLLECTION

Avicula crocea Lamarck, 1819

Lamarck, 1819: 148, no. 6; Dunker, 1872: 35, 36, pl. 11, figs. 1–4.

Type locality: "Habite les mers de l'Isle de France pour ler coquilles [a et b]. Mus. n°. La variété [c] ne m'est pas connue" (Lamarck, 1819: 148).

Figured specimen: LMD/LÖB 132968a, one pair of disarticulated valves; dimensions (RV): hinge length 62.5 mm, height 29.7 mm, length 59.5 mm.

Comments: The specimen from LMD/LÖB 132968a closely corresponds to figs. 1 and 2 by Dunker (1872: pl. 11; fig. 1 showing the exterior of RV superimposed over the interior of LV; fig. 2 showing the exterior of LV). The specimen perfectly matches the illustrations in overall shape, size, color, and in a unique pattern of radial dark chestnut rays in both valves and the pattern of the abraded dorsal part of RV. No historical labels are preserved. Dunker's fig. 3 is based on a specimen from the Cuming collection from Island of Mindoro, Philippine Islands, previously illustrated by Reeve (1857: pl. 15, fig. 57). The source of fig. 4 is not determined.

Avicula (*Meleagrina*) *flabellum* Reeve, 1857

Avicula flabellum Reeve, 1857: Species 7, pl. 5, figs. 7, 8, pl. 7, fig. 16; Dunker, 1872: 20, 21, pl. 6, fig. 1.

Type locality: "Venezuela" (Reeve, 1857).

Figured specimen: ZMB 112.822, one pair of disarticulated valves; dimensions (RV): hinge length 32.7 mm, height 45.0 mm, length 42.3 mm.

Comments: Avicula flabellum was based on the Venezuelan specimens from the Cuming collection (Reeve, 1857: species 7). To illustrate Reeve's species, Dunker used a shell from Porto Cabello, Venezuela, that was communicated by Tams (Dunker, 1872: 21). ZMB 112.822 contains a disarticulated shell with the enclosed byssus from the Dunker collection that perfectly matches an articulated shell illustrated by Dunker (1872: pl. 6, fig. 1) in overall shape, size, and color, and several unique features: (1) multiple small epibionts (that appear as small white dots in the figure) cemented proximal to the umbo; (2) the presence of the byssus; and (3) the long pale brown and dark brown scales of the exterior surface. Three labels accompany the ZMB lot:

Label 1: "flabellu / Reeve / Porto Cabello / Tams / Dk 6.1 / nicht genau ["not exactly"; here translated; lines 1–4 handwritten in pencil by Dunker; lines 5 and 6 handwritten in ink by Martens];
Label 2: "coll. Dunker" [printed];
Label 3: "Zoologisches Museum Berlin. [printed] / Porto Cabello Dunker" [handwriting unidentified; typical ZMB label].

Avicula heteroptera Lamarck, 1819

Lamarck, 1819: 148, no. 4; Dunker, 1879: pl. 24, fig. 8; 1880: 70, 71.

Type locality: Unknown.

Figured specimen: ZMB 112.863, single LV, posterior extremity of posterior auricle damaged; dimensions (LV): hinge length 45.3 mm, height 17.3 mm, length 25.0 mm.

Comments: The specimen from ZMB 112.863, a single LV, perfectly matches the illustration by Dunker, by the overall shape, size, and color pattern, and, more specifically, by a notch-like damage of the ventral border of the anterior auricle and the outline of the white encrusted area at the umbonal region. Two labels accompany the ZMB lot:

Label 1: "Tab. 24 f. 8 / T. 24" [handwritten by Dunker];

Label 2: "heteroptera. [handwritten in pencil by Dunker] / Delessert recueil 14, 7 / Dunk monogr Tf 24 fig 8!" [handwritten in ink by Martens].

Avicula iridescens Reeve, 1857

Reeve, 1857: Species 48, pl. 13, fig. 48; Dunker, 1872: 37, pl. 11, fig. 5.

Type locality: "Moluccas" (Reeve, 1857).

Figured specimen: LMD/LÖB 124268a, one pair of disarticulated valves, extremities of posterior auricles of both valves broken off; dimensions (RV): hinge length 72.4 mm, height 41.0 mm, length 57.6 mm.

Comments: The specimen upon which the species was based derives from the Cuming collection (Reeve, 1857: Species 48). Dunker (1872: 37) stated that, according to Cuming and Gruner, the species is native to the Moluccas. The lot from LMD/LÖB 124268a contains a specimen from the Gruner collection that perfectly matches the figured shell in overall shape, size, and several unique features: (1) the white incrustation of the exterior surface of the auricle of RV; (2) a notch on the ventral margin of RV; and (3) a yellow arc-shaped stripe on the interior nacreous surface of the posterior auricle of LV. Five labels accompany the LMD lot:

Label 1: "Gruner 1328 b / Avicula / crocea var. Lam. / Ostindien" [handwriting unidentified];

Label 2: "Gruner 1328 b / Avicula / crocea variet. ? ? / Ostindien / (nach Dunker sehr zweifelhaft) ["(very dubious according to Dunker)"; here translated]" [handwriting unidentified];

Label 3: "Avicula / crocea / var. b. Lamk" [handwritten by Dunker];

Label 4: "Diese Art / ist mir noch zwei / felhaft ["This variety / still appears dubious to me"; here translated]" [handwritten by Dunker];

Label 5: "Avicula / crocea Lam. Var. / Ost Indien / Gruner / 1 [handwriting unidentified] Löbbecke-Museum [printed]";

Label 5: "1328" [printed; affixed to posterior auricle of LV].

Avicula (*Meleagrina*) *margaritifera* (Linnaeus, 1758)

Mytilus margaritiferus Linnaeus, 1758: 704, no. 209 [the Indian Ocean]; Dunker, 1872: 4, 7–9, 13, 27, pl. 1, fig. 2, pl. 3, fig. 1 [juv.], pl. 9, figs. 2, 3; 1879: 58, 59, pl. 20, figs. 1, 2 [var.]; 1880: 75, 78, pl. 26, fig. 1. Note: Dunker (1872: 7) erroneously refers to Figures 1 and 2 on Plate 9 (instead of Figures 2 and 3 respectively).

Type locality: "Habitat in utriusque Indiæ oceano" (Linnaeus, 1758: 704).

Figured specimens: ZMB 112.799a, one pair of articulated valves; dimensions (RV): hinge length 27.0 mm, height 27.7 mm, length 30.0 mm; ZMB 108.682, one pair of disarticulated valves, anteroventral margins of both valves damaged; dimensions (RV): hinge length 33.9 mm, height 54.3 mm, length 45.2 mm; ZMB 112.813, single RV; dimensions (RV): hinge length 82.5 mm, height 88.3 mm, length 89.2 mm.

Comments: The sources of Figure 2 (Plate 1), Figure 1 (Plate 3), and Figure 2 (Plate 20) were not determined. Figure 1 on Plate 26 is reproduced from Chemnitz (1785: pl. 80, fig. 718).

The specimen illustrated by Dunker (1872) on Plate 9, Figure 2, perfectly matches one of the two conspecific specimens from a lot from the Dunker collection, ZMB 112.799, in shape, color, and size, and a several unique features: (1) the number and unique pattern of green radial rays; (2) green color posterior auricle in RV; and (3) the green color of the exposed part of the inner surface of the anterior shell margin close to the byssal notch. The shell is articulated as depicted. Three labels accompany ZMB 112.799:

Labels 1: "*M. margaritifera* Linn. / (young.). Hbf" [handwriting unidentified];

Label 2: "Landauer" [handwritten by Dunker];

Label 3: "coll. Dunker" [printed; glued to verso of Label 2];

Label 4: "Zoolog. Museum Berlin. [printed] / Dunker" [handwritten by Martens].

The specimen illustrated by Dunker (1872) on Plate 9, Figure 3, perfectly matches a specimen from the Dunker collection, ZMB 108.682, in shape, color, and size, and a number of unique features: (1) the number and the pattern of green radial rays; (2) a dorsally curved extremity of the anterior auricle of RV; and (3) the abrupt color change of the radial rays from green to black in the middle of RV. The specimen is shown articulated. The anterior shell margin of RV and many scales along the ventral margin of RV are damaged. The specimen was initially misidentified by Martens as the type of *A. cochenhauseni*, probably because Dunker (1872: 7) mistakenly referred to the shells illustrated on Plate 9 as Figures 1 and 2 instead of Figures 2 and 3 (see the note above and the entry for *Avicula cochenhauseni*). Later in the test, however, the figures cited properly (Dunker, 1872: 27).

Two labels accompany ZMB 108.682:

Label 1: "menstr? [?] / ⁜ / *Cochenhauseni* Dkr" [handwritten by Martens];

Label 2: "Avicula / cochenhauseni Dkr ⁜ / Dunk Avic. tf 9 fig 3 / China v Cochenhausen" [handwritten by Martens].

The shell of the variety of *A*. (*M*). *margaritifera* illustrated by Dunker (1879: pl. 20, fig. 1) perfectly matches the specimen from the Dunker collection, ZMB 112.813, in shape, color, size, and several unique features: (1) the pattern and relative length of radial dark brown rays; (2) the outline of the abraded outer shell layer in the umbonal regions; and (3) the outline of extensive damage along the ventral margin, particularly at the posteroventral region. The illustration shows only a single RV, corroborating the absence of LV.

Four labels accompany ZMB 112.813:

Label 1: "~~margaritifera~~ / *capensis* Sow / Dunker monogr. / 20, 1! S . 58" [handwritten by Martens];

Label 2: "*Margaritifera* / *margarit*. var. / Port Natal. / Heynemann." [handwritten by Dunker];

Label 3: "coll. Dunker" [printed; glued to verso of Label 2];

Label 4: "Zoologisches Museum Berlin. [printed] / Port Natal Dunker" [handwritten; standard ZMB label].

Avicula physoides Lamarck, 1819

Lamarck, 1819: 149, no. 12; Dunker, 1872: 25, pl. 8, figs. 2, 3 (var.).

Type locality: "Habite les mers du nord de la Nouvelle Hollande, sur des sertulaires, des plumulaires, etc." (Lamarck, 1819: 149).

Figured specimens: ZMB 112.868a, one pair of disarticulated valves, extremity of posterior auricle damaged; dimensions: hinge length 11.7 mm, height 10.8 mm, length 12.5 mm; ZMB 112.869a, one pair of disarticulated valves, anterior and ventral margins slightly damaged; dimensions: hinge length 10.8 mm, height 8.7 mm, length 9.2 mm.

Comments: To illustrate a variety of *Avicula physoides* Lamarck, 1819, Dunker used specimens from Australia communicated by Dr. Preiss (Dunker, 1872: 25). Two ZMB lots contain specimens from the Preiss collection that closely resemble the shells depicted by Dunker (1872: pl. 8, figs. 2, 3), as was previously recognized by Martens.

ZMB 112.868 contains a larger pair of disarticulated valves and a smaller single LV. The specimen with both valves resembles the figured shell by Dunker (1872: pl. 8, fig. 2) in overall shape, size, and color, although, admittedly, the illustrated shell is of darker green and somewhat generalized. Three labels accompany ZMB 112.868:

Label 1: "Avic. Papilio- / nacea, Lam. / (Mke.) Austr. / Dr. Preiß. 12" [handwritten by Dunker; original label of the Dunker collection];

Label 2: "physoïdes / Dkr / Austr / taf 8. fig 2." [handwritten by Martens];

Label 3: "coll. Dunker" [printed; glued to verso of Label 1].

ZMB 112.869 contains two pairs of disarticulated valves. One of the specimens resembles the figured shell by Dunker (1872: pl. 8, fig. 3) in overall shape, size, and non-typical for this species protruding posterior auricle. As is the case with the specimens in ZMB 112.868, the illustration is quite unspecific and does not have sufficient details to establish a definitive match with the specimens. The illustration might be an idealized composite image. Five labels accompany ZMB 112.869:

Label 1: "Avicula phy- / soïdes Lam. / (Mke. / Austr. / Dr. Preiß 8!" [handwritten by Dunker; original label of the Dunker collection];

Label 2: "Avicula papilionacea / Lam. / ad Novam Hollan / diam. / cel. Preiss." [handwriting unidentified];

Label 3: "Avicula virens / Lam. / ad Novam Hol- / landiam / cel. L. Preiss." [handwriting unidentified];

Label 4: "Dunker / Taf 8 fig ~~2~~. 3." [handwritten in ink by Martens; the number "2" crossed out in pencil by unidentified person];

Label 5: "coll. Dunker" [printed; glued to verso of Label 1].

Avicula tongana (Quoy & Gaimard, 1834)

Meleagrina tongana Quoy & Gaimard, 1833: pl. 77, figs. 14, 15; 1834: 460, 461; Dunker, 1880: 73, 74, pl. 25, fig. 5.

Type locality: "Habite Tonga-Tabou" (Quoy & Gaimard, 1834: 461).

Figured specimen: ZMB 112.870a, single LV; hinge length 21.7 mm, height 24.3 mm, length 27.3 mm.

Comments: One of two specimens in ZMB 112.870 matches the illustration by Dunker

(1880: pl. 25, fig. 5) in overall shape, color, size, and in two unique features: a little notch at posterior end of the dorsal margin and a light-colored damaged area of the posterior margin. Moreover, only LV is illustrated, which is consistent with the absence of the matching RV. This lot also contains a single RV of a much smaller specimen (approximately 10 mm hinge length) that might not be conspecific with the illustrated specimen. Three labels accompany the ZMB lot:

Label 1: "Meleagrina ton. / gana Q et G. III. / 460. t. 77. f. 14. 15! / Avic. Viridis Ch." [handwriting unidentified] "/ Dinke 25.5!" [handwritten by Martens];
Label 2: "coll. Dunker" [printed; glued to verso of Label 1];
Label 3: "Tongana / Q. G." [handwritten by Martens].

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SHELL BANDING POLYMORPHISM IN *CEPAEA VINDOBONENSIS* IN RELATION TO HABITAT IN SOUTHEASTERN POLAND

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ABSTRACT

We studied the frequencies of shell-banding variants of *Cepaea vindobonensis* (Férussac) in open, semi-open and shaded habitats in two geographically separate but climatically similar areas in southeastern Poland. The faint-banded phenotype was more frequent in open and semi-open habitats than in shaded areas. Within the dark-banded phenotype, those with missing bands were associated with open and semi-open habitats, and those with fusion of bands with shaded habitats. The feature most strongly associated with habitat type was the width of bands relative to the ground area. It was significantly higher in shaded habitats than in semi-open areas, in which it was significantly higher than in open habitats. Our study thus indicated the effect of local microclimatic selection on the frequencies of various phenotypes, resulting in light shells being more common in open habitats and dark shells in shaded areas. This association of shell characteristics with habitat type was more pronounced in the area where *Cepaea vindobonensis* inhabited more natural habitats and showed higher polymorphism.

Key words: color, climatic selection, land-snails, microclimate, natural selection, pigmentation, rate of evolution, *Cepaea*.

INTRODUCTION

Cepaea vindobonensis (Férussac, 1821) is a helicid land snail inhabiting southeastern Europe; its continuous distribution ranges from the northern Caucasus to the Balkans, Appenine Peninsula, eastern Austria, Czech Republic, and southeastern Poland (Kerney et. al., 1983; Riedel, 1988). It is a xerothermophilic species, usually inhabiting open and exposed habitats. In the central and southern parts of its distribution it also occurs in woods and other tree-covered sites (Rotarides, 1926; Jones, 1975; Dhora, 1985; Kramarenko et al., 2007). Polymorphism in this species differs from the extensive polymorphism in the congeners *Cepaea nemoralis* (Linnaeus, 1758) and *Cepaea hortensis* (Müller, 1774) in that the ground color of the shell is always yellowish to white, and the usual number of bands in most populations is five. Bands may be missing or fused in some populations. Two major forms can be distinguished: dark-banded and faint-banded, with dark-brown-colored and straw-colored bands, respectively. In *C. nemoralis* and *C. hortensis*, the ground color of the shell, presence or absence of bands, number of bands on a banded shell and fusion of bands are genetically controlled (Jones et al., 1977). No data on the formal genetics of *C. vindobonensis* are available, but by analogy to other *Cepaea* species genetic control of the shell banding polymorphism may be assumed. There are only a few studies relating shell polymorphism to environmental factors in *C. vindobonensis*; most suggest an effect of climate. In the Czech Republic (Honěk, 2003), the faint-banded morph occurs almost exclusively south of the 17°C isotherm of mean June temperature, and the increase in frequency of dark-banded morph from south to north parallels the decrease in average April–August temperature and sunshine hours. Also, populations in open, grassy habitats tend to include pale morphs, and these are at higher frequencies than in dense forb stands (Honěk, 2003). In Greece, in a hot and dry area near the Axios River, the faint-banded morph reaches 30%, in contrast to the cooler and more humid region of Logos, where it is extremely rare (Staikou, 1999). In Croatia, morph frequencies are associated with topography, which can be readily explained by climatic selection (Jones, 1973, 1974).

In these previous studies, variation was analyzed only as faint-banded and dark-banded. However, within the typical five-banded form, the width of individual bands can vary considerably, resulting in variation in the proportion of the shell covered with bands, which can be compared among populations. Such an approach was first used in the largely forgotten study of Rotarides (1926); we also used it here.

In *Cepaea*, climatic selection can affect morph frequencies on a large geographic scale (Jones et al., 1977) and on the scale of habitats (Ożgo, 2005). In this study, we related the polymorphism of *C. vindobonensis* to habitat type in two geographically distant but climatically similar areas in southeastern Poland. This allowed us to study the effect of similar selective forces on populations influenced by different historical factors.

MATERIAL AND METHODS

The study was carried out in two areas in southeastern Poland: the Hrubieszów area and Sandomierz area (Fig. 1), both at ap-

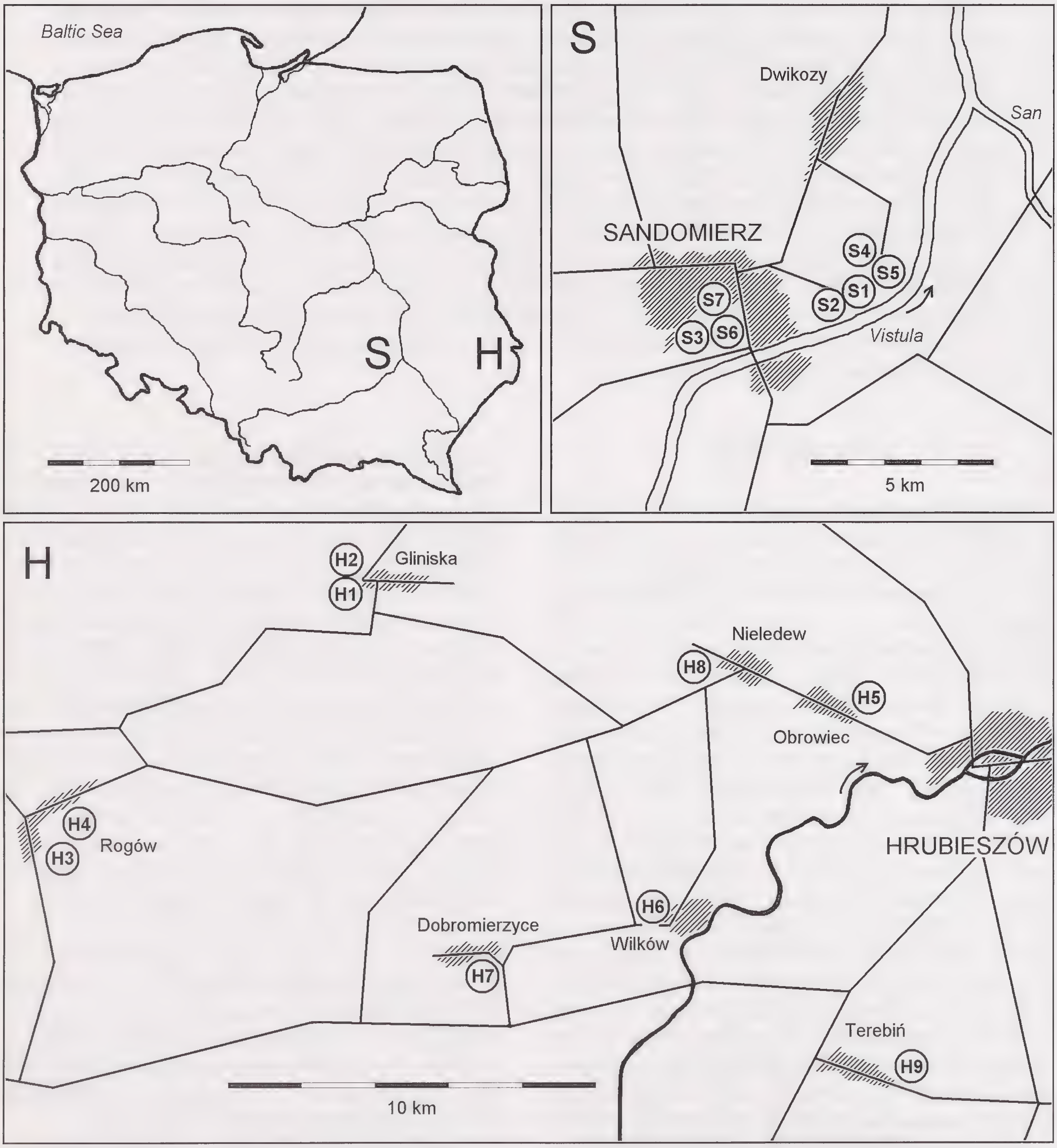

FIG. 1. Maps of Poland showing the location of the study sites. H1–H9, populations in the Hrubieszów area; S1–S7, populations in the Sandomierz area.

proximately 50°N and 200 m a.s.l, but 140 km apart. Climatic characteristics of the areas are similar: in the Hrubieszów area, average annual temperature is 7.2°C, average May–September temperature is 15.2°C, and average July temperature is 17.4°C; annual precipitation is 563 mm, and precipitation in May–September is 340 mm. In the Sandomierz area these values are 7.6°C, 15.6°C and 17.8°C, 568 mm and 339 mm, respectively (data for 1961–1990 from the Institute of Meteorology and Water Management, Kraków). In the Hrubieszów area, the snails occurred in fairly stable populations inhabiting remnants of natural habitats, whereas in the Sandomierz area they occurred mostly in more disturbed semi-natural habitats in urban and suburban environments.

We collected samples in 2002–2005 from areas of uniform vegetation. The collection sites were as small as practically possible and usually did not exceed 1,000 m^2. We collected live snails and well-preserved dead shells, and only mature individuals with a well-developed lip. We included only sites where we obtained samples of 30 or more, for a total of 1,032 individuals from 16 populations. Populations of adequate size were hard to find.

We divided the habitats into three categories: open, semi-open, shaded. Open habitats included escarpments covered with short vegetation and xerothermic meadows. Shaded habitats were those with trees dense enough to give shade throughout the day. Semi-open sites were intermediate in character, with shrubs or scattered trees; places where snails occurred only at the edge of a wooded area were included in this category.

We first scored the shells as faint-banded or dark-banded. Bands in the faint-banded morph are often blurred and spread into areas of whitish coloration, giving the shell an overall yellow appearance; we therefore did not score these shells further. Within the dark-banded phenotype, we scored missing and fused bands, and we estimated the proportion of the area of the shells covered with bands. Within dark-banded shells with all bands present and separate, we estimated the relative width of consecutive bands (Rotarides, 1926). First, we measured the width of the bands and of the ground area to an accuracy of 0.5 mm, and calculated the percentage of the shell covered with bands (%B). We took the measurements at a line across the body-whorl at right angles to the lower lip of the mouth, as is standard in scoring band fusion in *Cepaea* species (Cain & Sheppard, 1950). As noted by Rotarides (1926), the accuracy of 0.5 mm is optimal; greater accuracy would not improve the results because of the frequent smudging and spreading of the bands. Second,

TABLE 1. Composition of *C. vindobonensis* samples. Missing bands – number of shells with any bands missing in the dark-banded phenotype; Fused bands – number of shells with any bands fused in the dark-banded phenotype; Average %B – average proportion of the area of the shells covered with bands in the dark-banded phenotype. Habitats: O – open; I – intermediate; S – shaded.

Sample number	Total snails	Faint banded	Dark banded	Missing bands	Fused bands	Average %B	Habitat
H1	98	76	22	6	0	29.4	O
H2	67	23	44	7	1	31.4	I
H3	71	0	71	3	0	34.9	S
H4	51	1	50	1	0	35.3	O
H5	49	10	39	0	0	36.5	O
H6	100	9	91	1	4	37.5	O
H7	79	0	79	0	16	48.1	S
H8	63	5	58	0	15	50.2	S
H9	57	12	45	0	18	50.6	S
S1	46	7	39	0	0	37.6	O
S2	56	6	50	0	0	39.1	O
S3	98	2	96	3	0	41.6	O
S4	31	0	31	0	0	42.8	I
S5	30	8	22	0	0	45.1	I
S6	65	0	65	0	1	46.1	S
S7	71	1	70	0	0	46.7	S

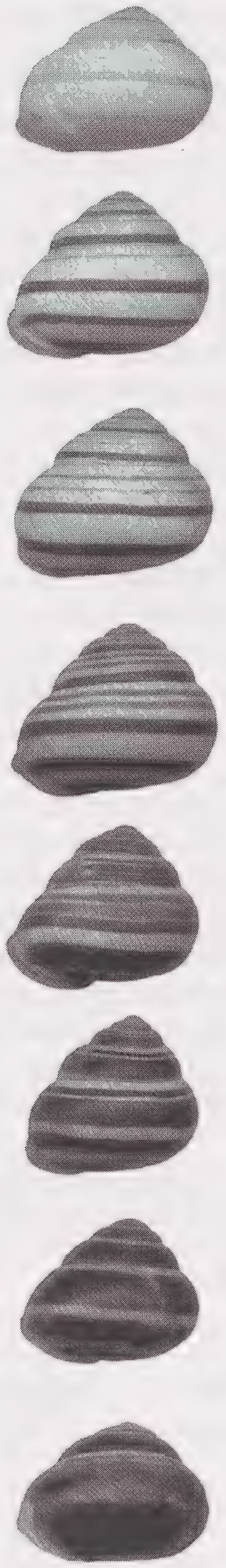

FIG. 2. The range of variation of *C. vindobonensis* shells observed in this study. From top to bottom: faint-banded shell; dark-banded shell with band 2 missing - 10345; dark-banded shell with very narrow bands – 1 = 2 < 3 = 4 < 5; dark banded shell with bands of medium width – 1 = 2 < 3 = 4 = 5; dark-banded shell with wide bands – 1 < 2 > 3 < 4 > 5; dark-banded shell with fused bands – 1(23)45; dark-banded shell with fused bands – (123)45; dark-banded shell with fused bands – (12345).

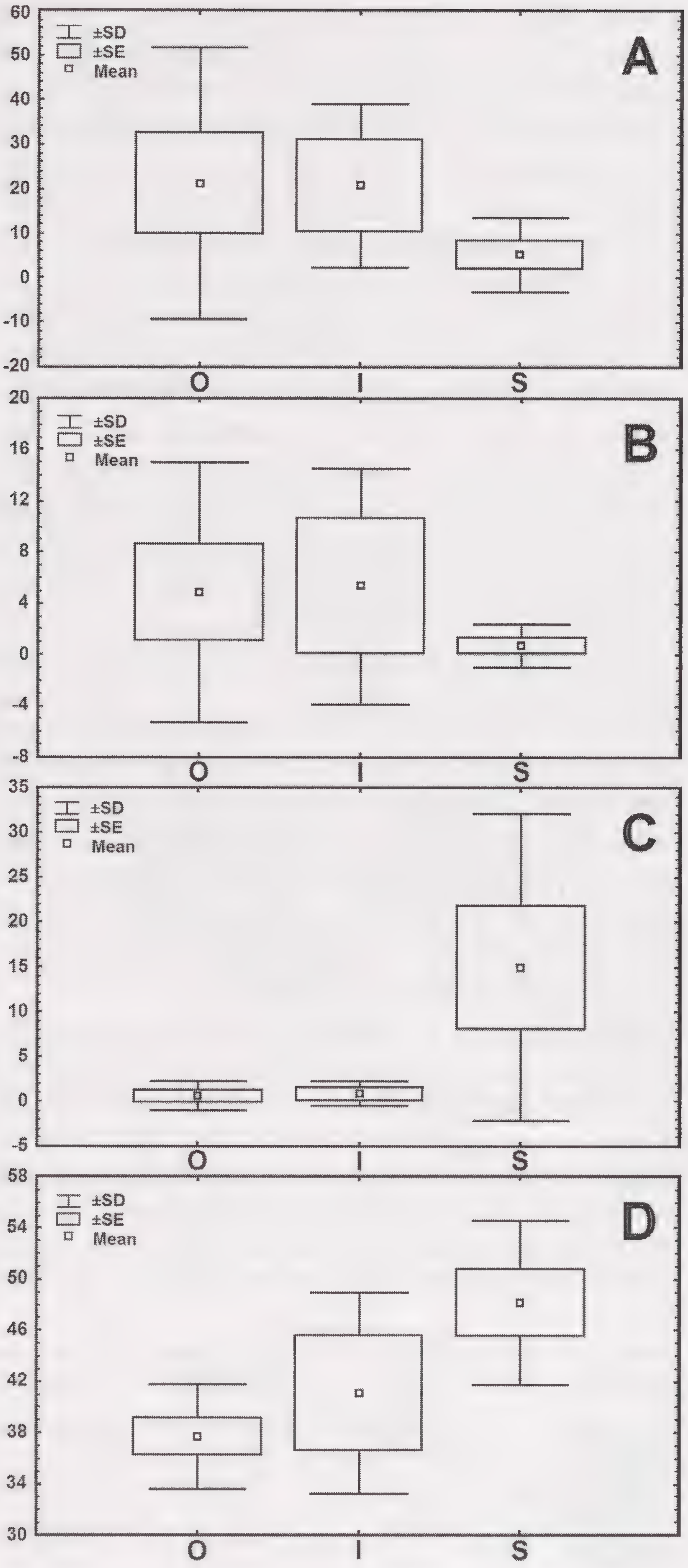

FIG. 3. Association of the frequencies (%) of the major *C. vindobonensis* morphs with habitat. A – faint-banded shells, B – shells with missing bands, C – shells with fused bands, D – the proportion of shells covered with bands (%B); O, open habitats n = 7; I, intermediate habitats n = 3; S, shaded habitats n = 6; SD, standard deviations; SE, standard errors. Percentage values arcsin transformed.

we compared the width of each band to that of its neighbors; 1 = 2 = 3 = 4 = 5 designates a shell with equal width of all bands, while 1 < 2 > 3 = 4 = 5 indicates that the first band is narrower than the second, which is wider than the third; theoretically in shells with all five bands present and separate, there are 81 possible patterns (Rotarides, 1926).

RESULTS

The composition of the samples is given in Table 1. Figure 2 illustrates the observed range of variation of *C. vindobonensis*, and Figure 3 shows variation in frequencies of shell variants in each habitat type. The faint-banded phenotype occurred in both study areas and in most populations. It was more frequent in open and intermediate habitats than in shaded areas, but the difference just failed to reach significance (Mann-Whitney test, p = 0.056). Shells with fused or missing bands within the dark-banded phenotype were much rarer than faint-banded shells and occurred almost exclusively in the Hrubieszów area. Shells with missing bands were associated with open and intermediate habitats, in which 86% of them occurred (sites H1, H2, H4, H6, and S3), while only three individuals came from a shaded habitat (H3 – but see Discussion). Differences in frequency among habitat types were, however, not significant, probably because of the small overall number of such shells (n = 21). Shells with fused bands occurred mostly (91%) in shaded habitats (sites H7, H8, H9, and S6), while only four individuals came from an open habitat (H6) and one individual from an intermediate habitat population (H2). The difference in frequency between shaded and other habitat types was statistically significant (Mann-Whitney test, p = 0.046). Thus, although only one of those three variants shows a formally significant relationship to habitat, all three show the trend expected from earlier work, both in median values and in the proportion of sites in which the variants occur.

The width of the bands relative to the ground area, that is, the proportion of the shells covered with bands (%B), showed the strongest association with habitat type. The average %B was higher in shaded than in intermediate habitats, in which it was higher than in open areas (Fig. 3 D), (Kruskal-Wallis test, p = 0.049).

In Figure 4 sites are arranged in order of increasing average %B. Significance of differences among the sites was established with the nonparametric multiple comparisons test with unequal sample sizes (Zar, 1999). Populations in group 1 do not differ significantly from each other, but all are significantly different from those in group 2, which again do not differ significantly amongst themselves. In the mid range (S1 to S4 inclusive), S2 and S3 differ from both extremes, but not from all members of either group; S1 and S4 differ from one or the other extreme, but not from all members of the group to which it belongs. Most shaded habitats belong to group 2, confirming the result above: differences between shaded habitat populations of group 2 and every population of group 1 are all highly significant (p < 0.001).

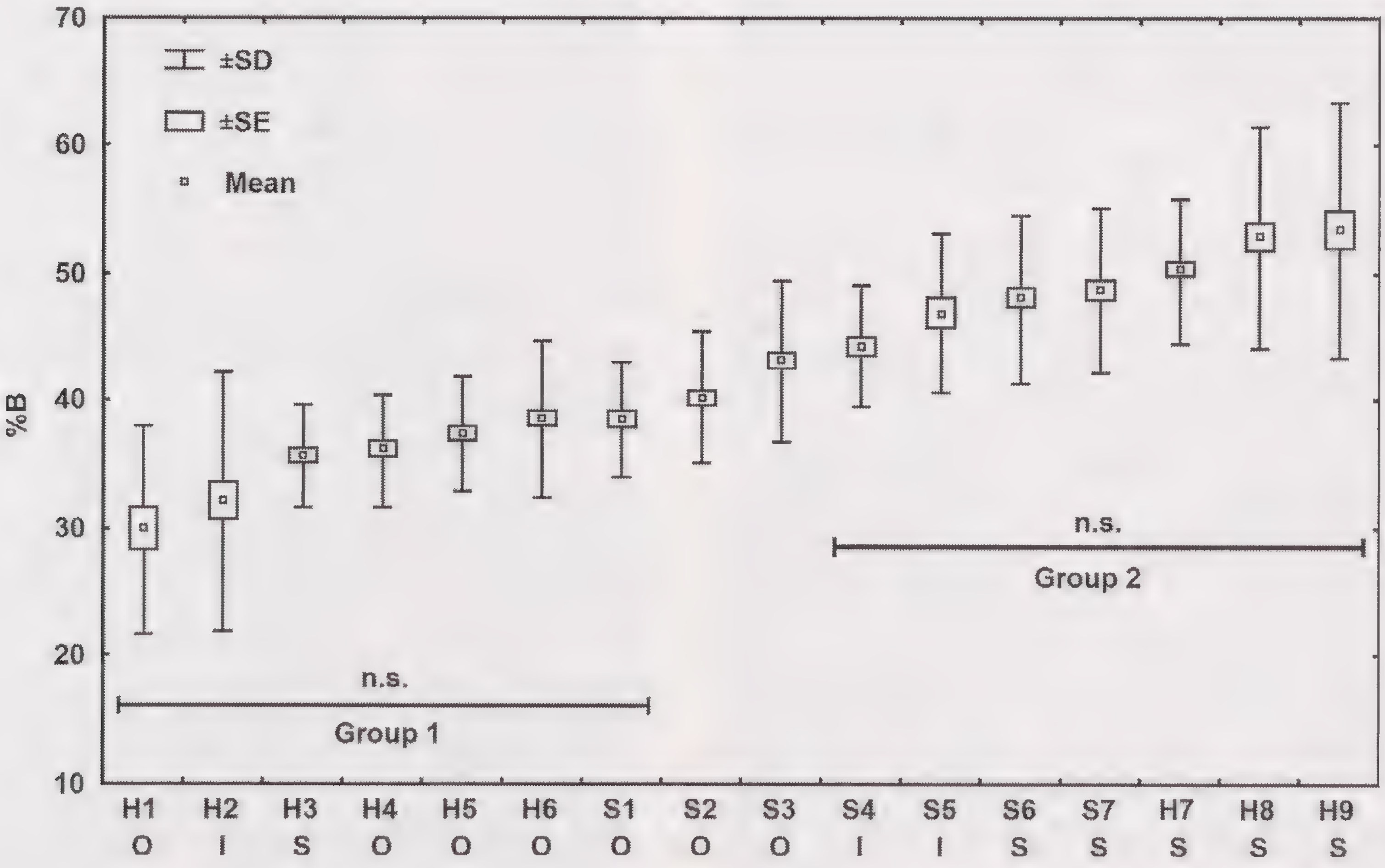

FIG. 4. Mean values, standard deviations (SD), and standard errors (SE) of the proportions of shells covered with bands (%B) in *Cepaea vindobonensis* populations, percentage values arcsin transformed. H1–H9, populations in the Hrubieszów area; S1–S7, populations in the Sandomierz area; O, open habitats; I, intermediate habitats; S, shaded habitats; n.s., no statistical significance in the nonparamentric multiple comparisons test.

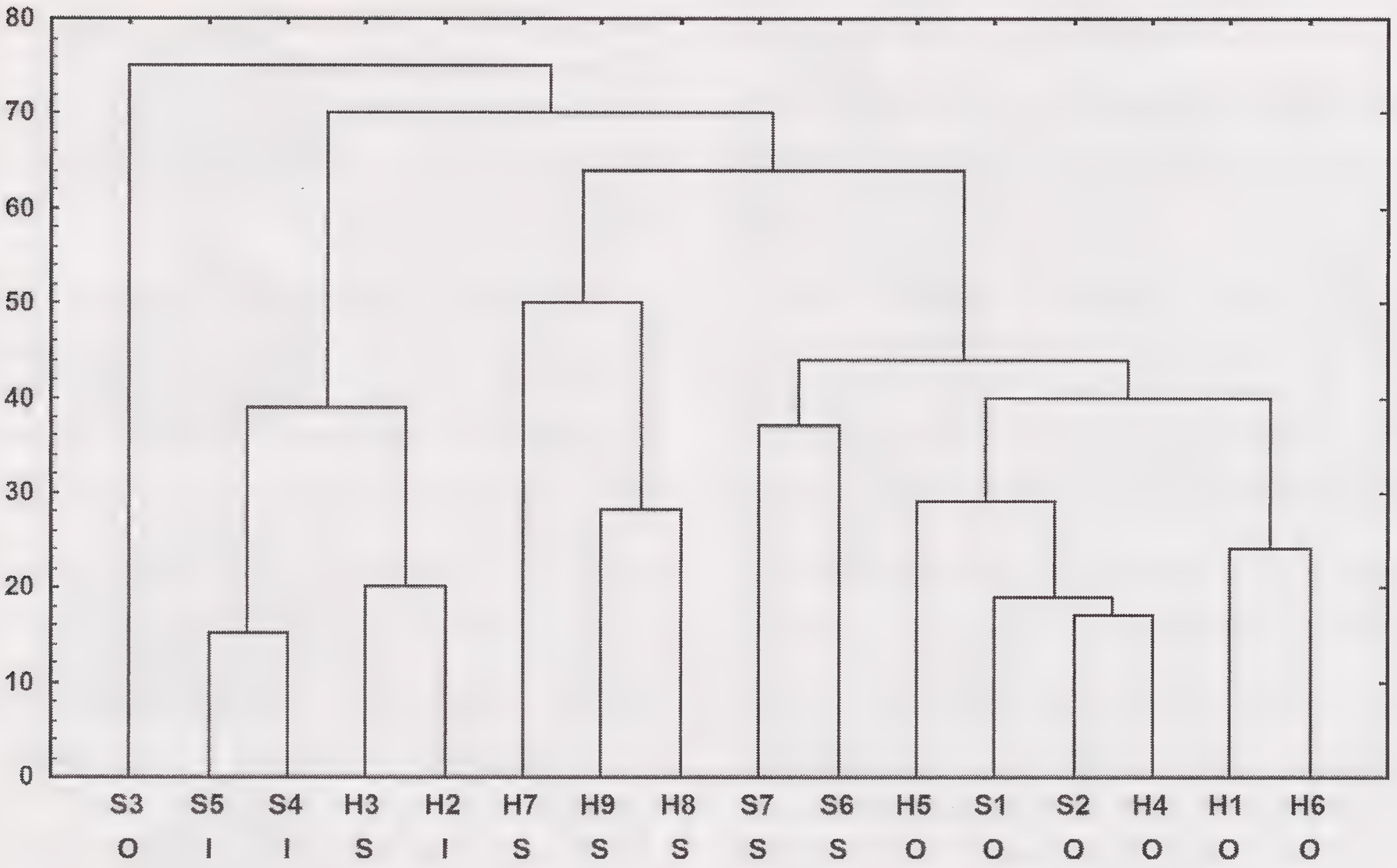

FIG. 5. Dendrogram of percentage of similarity affinities between sites. H1–H9, populations in the Hrubieszów area; S1–S7, populations in the Sandomierz area; O, open habitats; I, intermediate habitats; S, shaded habitats.

Populations in the Hrubieszów area exhibit more extreme values of %B than populations in the Sandomierz area, which group together in the middle of the graph. The anomalous position of H3 is considered below.

The width of consecutive bands on a shell revealed 25 banding variants. The two commonest (1 = 2 < 3 < 4 < 5 and 1 = 2 < 3 = 4 <5) occurred at all sites reaching an overall frequency of approximately 30% each, and within each there was the same trend for a greater percentage of band cover in shaded habitats (data not shown).

Frequencies of other variants varied considerably among populations, and all variation has been used to examine the similarity in occurrence of the 25 variants of relative band thickness among sites using the percentage of similarity (Southwood, 1968). Figure 5 shows the dendrogram of affinities among sites. Populations from open and intermediate habitats cluster together with only one "mismatch" (site H3 – but see Discussion). Within these clusters populations from both study areas are present. Populations from shaded habitats also cluster together, but form two groups connected at a low affinity level; those groups are consistent with geographical location. One open habitat population (S3), geographically close to S6 and S7, is very distinct and does not cluster with any of the rest.

DISCUSSION

Shell variation in *C. vindobonensis* is mainly in band pigmentation and cover. Some of this variation is continuous; its heredity is unknown, and the term polymorphism is used only for convenience. Variation in the darkness of the shells results from the color of the bands, and in the dark-banded phenotype from the number of missing or fused bands and from their width. Although the small number of samples available has restricted our ability to detect significant differences between habitats, the results of our study show that light shells were more common in open habitats and dark shells in shaded areas, which is consistent with the predictions of climatic selection.

Dark-banded *C. vindobonensis* placed experimentally in direct sunlight reach a significantly higher equilibrium temperature than faint-banded individuals of the same size (Jones, 1973; Staikou, 1999). In *Cepaea nemoralis*, five-banded phenotypes, especially those with fused bands, reach significantly higher internal temperatures (Heath, 1975), reflect significantly less light, lose more weight, cease activity earlier (Chang, 1991), and suffer more from differential mortality in direct sunshine than the unbanded phenotypes (Richardson, 1974). Unbanded shells are extremely rare in *C. vindobonensis*, but within the five-banded phenotype, the width of bands varies considerably resulting in large variation in the general darkness of the shells. This presumably affects such features as spectral reflectance, internal temperatures, weight loss and activity. In *Helicella candicans* (Pfeiffer, 1841) (synonyms: *Helicella obvia, Xerolenta obvia*), which exhibits similar polymorphism to that in *C. vindobonensis*, shells with wide and fused bands have a higher rate of heating and reach equilibrium temperatures up to 2.5°C higher than shells with narrow bands (Honěk, 1993). In this species, dark shells are more common in areas with frequent fogs and greater cloudiness, and shells in habitats with dense and tall vegetation tend to be darker than those in short grass steppes (Honěk, 1993). In polymorphic populations of *C. nemoralis*, climatic selection affects mostly the ground color of the shells (Jones, 1973; Ożgo, 2005). However, in introduced populations with only yellow shells, it is the presence and fusion of bands that respond to local climatic selection: shadier habitats have higher proportions of banded shells and band fusions than sunnier ones (Richards & Murray, 1974). In this case, the polymorphism of *C. nemoralis* is analogous to that of *C. vindobonensis*, and the response to local climatic variables is similar.

Our results agree with those of Rotarides (1926) from Hungary. He compared two populations of *C. vindobonensis*; in the wetter and cooler wood, the shells were significantly darker than in the warmer and drier area. He gave an adaptive explanation, relating the properties of the shells to the microclimatic characteristics of the habitats. It is worth noting that his paper was published at a time of general consensus that visible variation in *Cepaea* was non-adaptive (Cameron, 1997), and a quarter of a century before the first widely recognized paper on the effect of selection on morph frequencies (Cain & Sheppard, 1950).

In our two study areas, the association of the proportion of shells covered with bands (%B) with habitat shadiness was similar, although more pronounced in the Hrubieszów area (Fig. 4). This may be because populations in the Hrubieszów area were more polymorphic for missing and fused bands – characteristics with which %B is strongly correlated (within the dark-banded category correlation between the frequency of shells with missing/fused bands and average %B in populations is $r = -0.656$, $p < 0.01$, and $r = 0.653$, $p < 0.01$, respectively). Hrubieszów populations inhabit more natural and stable habitats, suggesting a longer time for adaptation. These two explanations may be related to each other: greater variation in Hrubieszów may result from the greater habitat stability. In contrast, however, urban populations in southern Ukraine are more polymorphic than natural ones (Kramarenko et al., 2007). Differences between the Hrubieszów and Sandomierz areas may also have broader biogeographic causes. There are no major barriers between the Hrubieszów area and Ukraine, where shells with missing or fused bands are relatively common (Sverlova & Kirpan, 2004; Kramarenko et al., 2007). The Sandomierz area is on the other, western side of the Vistula River, a major geographic barrier. In populations of *C. vindobonensis* in western parts of the distribution of the species, missing or fused bands appear to be extremely rare (Honěk, 2003; our own unpublished data); possibly those two areas were colonized from different (eastern and western) refuges in the post-glacial period. It is also possible that the limited variation reflects some kind of founder effect, with Sandomierz populations carrying only a subset of the western range of variation.

The occurrence of the 25 variants of relative band thickness follows a habitat pattern (Fig. 5), but within shaded habitats two distinct geographical groups are present. This suggests some kind of selection. However, the genetic control of minor variants of shell banding is unknown, and there may be complex interactions between relative thickness of different bands and the proportion of the shell covered by them. The different results between areas for shaded habitats suggest an origin effect. As the habitat of *C. vindobonensis* is generally xerothermic, perhaps when confronted with the availability of more shaded habitats, the populations in the two areas increased their overall darkness in different ways, which is possibly just a chance effect.

Three populations need additional comment. The H3 population inhabited a wooded area, but its shell characteristics were typical of open habitats. However, the wood was a fairly recent one, developing after establishment of a nature reserve in 1965. The reserve was meant to protect xerothermophilic flora, but it was not managed and became overgrown with trees. The adaptation of *C. vindobonensis* appears to be much slower than the change in the habitat, and present shell characteristics of this population are possibly one of the few remnants of the original character of this place. This slow rate of adaptation contrasts with introduced *C. nemoralis* populations in southeastern Poland, in which significant shift in the frequencies of color and banding morphs in response to local climatic selection can occur in less than 20 years (Ożgo & Kinnison, 2008). Because the H3 population segregates with intermediate habitat populations in the dendrogram of banding pattern affinities, it may have already shifted in character towards more shaded habitats.

Populations H1 and H2 inhabited two sides of the same hill: a south-facing slope covered with short grasses (H1) and a north-facing slope with shrubs and sparse trees (H2). The history of this place is unknown, but it is likely that these populations share a common origin. Generally in the area of our study, the feature most strongly associated with habitat type was the proportion of the shells covered with bands (%B) in the dark-banded phenotype. This value was slightly but not signficantly higher in the northern (H2) than in the southern (H1) population (Mann-Whitney test, $p > 0.05$). However, these populations differed significantly in the frequency of faint-banded shells: 34% in the northern population, 78% in the southern population ($\chi^2 = 30.98$, $p < 0.001$). In this case, climatic selection apparently affected the frequencies of faint-banded shells, not the width of bands in the dark-banded shells.

Our results show that the *C. vindobonensis* shell polymorphism reacts to local climatic selection but, as exemplified by the H3 population, the adjustment proceeds relatively slowly. The major mechanism of adaptation is through the width of bands in the dark-banded shells, but in some circumstances it is the frequency of faint-banded shells that can be affected. The specific route depends presumably on initial gene-pools and/or the nuances of local selection.

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MOLLUSCS AND CLIMATE WARMING IN A LOW MOUNTAIN RANGE NATIONAL PARK

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ABSTRACT

Species restricted to the higher altitudes of low mountain ranges in Central Europe are among the species threatened. To quantify the influence of climate warming, we analysed the altitudinal distribution of mollusc species and modelled the occurrence and distribution of a high montane species under two scenarios of climate warming. Our analysis is based on samples of 3,437 individuals and 46 species collected at 111 localities. Number of individuals and species decreased with altitude. The slope of this decrease changed between 1,100 and 1,200 m a.s.l., which is the ecotone between mixed montane beech-fir-spruce and high-altitude spruce forests. In contrast to the majority of species and the number of species, the occurrence of *Semilimax kotulae* (Westerlund 1883), a Central European endemic, increased with altitude. Again, we found a change in the slope between 1,100 and 1,200 m a.s.l. Using geostatistical models of local temperature as well as spatial GLMs with Poisson and Gaussian errors, we modelled altitudinal distribution of diversity and species under consideration of environmental variables. Especially habitat age was an important predictor for the abundance of many species. For the high montane species *S. kotulae*, our model predicts a decrease in occurrence and abundance with global warming. Although global warming will lead to an overall increase in number of species, species occurring only at higher elevations, such as the *S. kotulae*, will probably become extinct within the study area.

Key words: altitudinal gradient, Bavarian Forest National Park, extinction risk, habitat tradition, *Semilimax kotulae*, BayesX.

INTRODUCTION

Global warming will lead to a latitudinal and altitudinal reorganization of distributional ranges (Parmesan et al., 1999; Parmesan & Yohe, 2003; Root et al., 2003). In extreme, these reorganizations may lead to a regional or even global extinction of species (Bakkenes et al., 2002; Berry et al., 2002; Thomas et al., 2004; IPCC, 2007a, b), and therefore the understanding of the implications of global warming is among the most important tasks for ecologists and conservationists (Sutherland et al., 2006). Despite considerable advances in our understanding of the relations between climate and species distribution at a global scale (Gaston & Blackburn, 2000; Gillooly & Allen, 2007), our knowledge of the local and regional effects of global warming is limited (Grabherr et al., 1994; Kappelle et al., 1999; Travis, 2002; Schrag et al., 2007).

High mountains show long altitudinal gradients that facilitate investigations on the upwards shifts of altitudinal distributions by climate warming (e.g., Kazakis et al., 2007; Pauli et al., 2007). However, the low mountain ranges of Central Europe, with altitudes less than 1,500 m, comprise in total the largest area of all mountains in Europe (CIPRA, 2007). Furthermore, they harbour a number of species with restricted distribution, some relicts from the ice ages (Limondin, 1992; Varga, 1995). Species restricted to the higher zones of these mountains seem to be vulnerable to local and regional extinctions by climate warming for two reasons (Parmesan, 2006): First, the low maximum altitude restricts the possibility to shift ranges upwards to escape the increase of tem-

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perature (Thomas et al., 2004; Thuiller, 2007). Second, the available area decreases with altitude. Any upwards shift leads to a decrease of habitats, to an increase in fragmentation and therefore to a decrease in population size with considerable risk of extinction by demographic stochasticity.

Community composition, species richness as well as location and size of distributional ranges of molluscs depend in particular on climate and soil (Sternberg, 2000; Tattersfield et al., 2001; Aubry et al., 2005; Kappes, 2005; Müller et al., 2005a). This close correlation of mollusc distribution and habitats is in part due to their low dispersal propensity (Waldén, 1981), and therefore molluscs should react very sensitive to global warming. Several species of molluscs are restricted to the upper zone of Central European low mountain ranges (Uminski, 1983). Most of these species are adapted to cold climates, and warming may force a shift of altitudinal distribution with the associated risk of extinction. Beyond this pessimistic scenario, the extinction of local populations may have negative genetic consequences for surviving species. The low dispersal propensity of molluscs leads to considerable genetic variation between populations (Marten et al., 2006). Similar to plants (Matthies et al., 2004), some of this variation may be adaptive (Calosi et al., 2008). The extinction of local populations will probably lead to a reduction in genetic diversity.

Several national parks were established to protect the unique fauna and flora restricted to higher altitudes of the low mountain ranges. The oldest of these national parks in Germany is the Bavarian Forest National Park. Climate warming, however, compromises the protection of high montane assemblages, a major conservation objectives in this and similar parks, of species. Even though the park authorities are unable to influence climate change, it is essential to understand the influence of global warming on the species and habitats for two reasons: Firstly, only if one has some knowledge about species turn-over due to global warming, one is able to detect additional threats. Secondly, the maintenance of habitat diversity is a possibility to decrease the extinction risk due to climate change. Therefore, it is necessary to understand the key resources of the species under consideration to mitigate the effects of climate change. Our study has therefore three main aims:

(1) To assess the influence of altitude and associated environmental variables on number of species and abundance of single species of molluscs in the Bavarian Forest National Park.
(2) To test the influence of site-specific habitat factors on diversity and occurrence of molluscs.
(3) To quantify changes in the extinction risk of high montane species with global warming.

METHODS

Study Area and Study Sites

The Bavarian Forest National Park is located in the southeastern corner of Bavaria, at the border to the Czech Republic (Fig. 1). The park covers approximately 24,000 ha at elevations from 650 m to 1,430 m a.s.l. Total annual precipitation is between 1,200 mm and 1,800 mm depending on altitude. Mean annual temperature (1970–2003) varies between 3.8°C and 5.8°C (Bässler, 2004). Geologically, the Bavarian Forest is the southwestern part of the Bohemian Massif, consisting of granite and gneiss and therefore acidic soils (Table 1). Depending on local conditions, above 1,150 m a.s.l. the vegetation is dominated by almost pure spruce *Picea abies* stands, with only a low proportion of beech, *Fagus sylvatica*, and mountain ash, *Sorbus aucuparia* (high montane forest). Below this, stands are dominated by a mixed montane forest of spruce, beech and fir, *Abies alba* (Walentowski et al., 2004). Due to infestation by bark beetles, mainly *Ips typographus*, large areas of forest have died back, and the resulting structure varies widely from stands with an open canopy, dominated by dead wood, to dense, closed stands (Müller et al., 2008).

We sampled molluscs as part of a more comprehensive program to characterise spatial variation of biodiversity. To get representative samples, we established four transects across the altitudinal range of the park. Along these transects we located a total of 293 plots with a distance of 100 m between adjacent plots. For the sampling of molluscs we randomly selected 111 plots that represented the major forest management types across the altitudinal gradient (Fig. 1). It is important to note that plots were not selected in respect to density or species richness of molluscs. Management types ranged from stands where trees infested by bark beetles are still removed, through stands where management by the forest authorities stopped with the establishment of the national park in 1970, to old-growth forest with veteran trees.

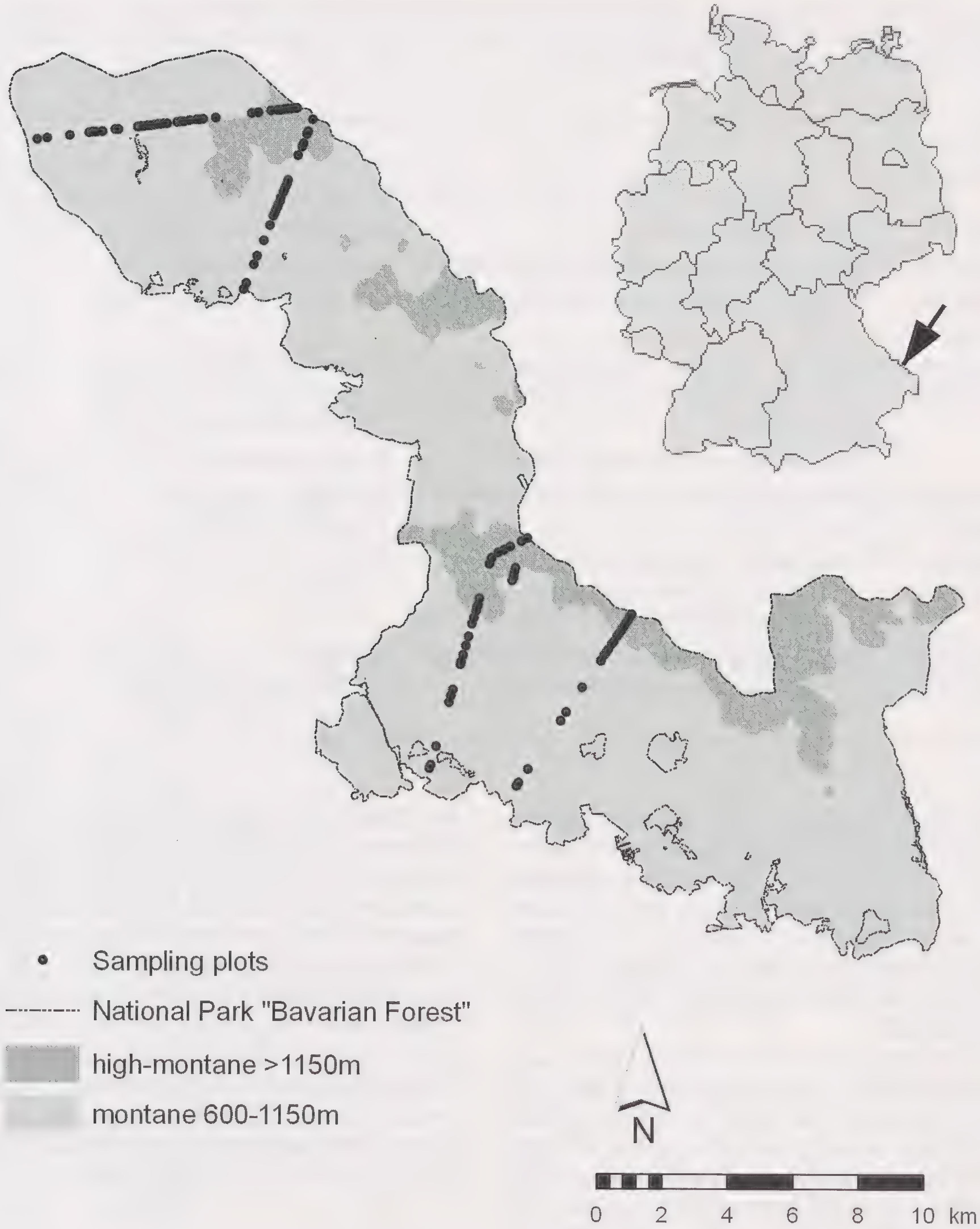

FIG. 1. Study area and location of the 111 plots used to sample molluscs along four altitudinal transects within the Bavarian Forest National Park.

Sampling Methods

Sampling during only one period of the year is not appropriate for all species of molluscs and, for example, early autumn appears to be the best time to sample montane molluscs (Uminski, 1975). Therefore, we used two methods. Firstly, installation of one pitfall traps for three weeks in July 2006 in the centre of each plot. Secondly, hand collection for 30 minutes of all individuals of live snails and slugs within a 0.1 ha area around the centre of each plot in September 2006. Sampling included a few small ephemeral water bodies within forest stands, where we found two *Pisidium* species. When it was not possible to identify an individual in the field, it was stored in 80% ethanol and identified in the laboratory using morphological characters (Wiktor, 1973; Kerney et al., 1983; Jungbluth et al., 1992; Wiktor, 2000). For

TABLE 1. Definition, source and measurement of the environmental variables used for the analysis of abundance, number of species and species richness of terrestrial gastropods and two *Pisidium* spec. within the Bavarian Forest National Park.

Environmental variable	Description	Source	Range total
Altitude	Height above sea level	Surface terrain model	655–1,419 m
Temperature	Mean annual temperature 2000–2007	ArcEcmo modelling	4.1–7.8°C
pH value of humus layer	pH value of humus layer (Bässler et al., 2008)	Laboratory analysis in 2006	2.3–4.42
Soil water balance index	Soil water balance calculated after Ewald et al. (2000) on a scale between 0 (dry) and 10 (wet)	Inspection of characteristics of the top soil as well as pH measurements in 2006	0.0–9.6
Age	Oldest tree in each sample plot	Forest inventory in 2002	49–400 years
Coarse woody debris	Amount of dead wood > 12 cm within 0.1 ha x 10	Inventory of plots along transects in 2006	0–708 m^3 ha^{-1}
Opening of canopy layer	Percentage of signals arriving at 1 m above ground averaged across the 0.1 ha sampling plots (Hyde et al., 2006)	Airborne laser scanning in 2006	0.0–96.8%
Plant species diversity	Shannon index of plant species (without moss) diversity in the field layer	Vegetation mapping in July 2006 using the Londo-scale (Londo, 1976)	0.0–3.13

all subsequent analyses, we totalled numbers of all individuals (abundance) and number of species across the two approaches. Species richness was estimated using the residuals from the regression of number of species versus individuals (Gotelli & Colwell, 2001). Technically, we used the number of individuals as a covariate.

For each plot, GPS coordinates were used to extract altitude from topographic maps and a terrain model of the park. To obtain reliable data for temperature and humidity, 30 data loggers were installed on representative sites across the altitudinal gradient during 2006. Additionally, we used data from five meteorological stations in the region that operated since 2000 to adjust the data collected in 2006 to the average between 2000 and 2007. Values for temperature are higher than those reported in our general description of the area, which refer to the period 1970–2003. With these data, we developed a model, using geostatistical modelling with ArcEGMO (Becker et al., 2002; Pfützner, 2002), to predict for each plot the mean annual temperature, using independent variables extracted from the terrain surface model (a detailed description is available on request by JM). These predicted values were used in all subsequent analyses. A complete list of environmental variables used for modelling the distribution of species is given in Table 1. Although precipitation is an important environmental factor for molluscs, we did not routinely consider precipitation during our study for three reasons. Firstly, we found a high co-linearity of temperature and precipitation (r = 0.71). Second, the scenarios of climate warming predict a decrease in precipitation by only 6% (Spekat et al., 2007). Third, for one species (*Semilimax kotulae*) we included beside temperature precipitation to check our results. However, conclusions were almost identical and therefore we concentrate on temperature.

Statistical Analyses

The plots of number of individuals and number of species *versus* altitude showed considerable scatter. However, a visual inspection suggested a clear pattern of the maximum values. Therefore, to visualize the trends with altitude, we used additive quantile regression smoothing (Koenker et al., 1994) as implemented in the package "quantreg" in R 2.6.0 (Koenker, 2007; $\tau = 0.8$).

To analyse the relationships of total number of individuals, the number of species, species richness and abundance of single species we used generalized linear models (GLM). For

TABLE 2. Results of spatial GLM for species which occurred within at least ten plots of the 111 sampled plots. Black triangles indicate significant influence (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), up means positive estimator, down negative estimator. To allow for direct comparisons of the estimators the predictors were standardised to a mean of zero and a variance of 1. Empty triangles indicate marginal significant effects ($p < 0.1$). Number of species is the number of species per sampling area whereas for the estimation of species richness we included number of individuals as a covariable. Altitudinal distributions of species given in Figure 2.

Dependant variable	Red data book	Frequency (occupied plots)	Temperature	pH-value of humus layer	Soil water balance index	Age	Coarse woody debris	Opening of canopy layer	Plant Diversity
Number of individuals without *S. kotulae*			▲ 10.0**	△ 2.6		▲ 6.9***			
Number of species			▲ 0.29*		▲ 0.10**	▲ 0.09**			▲ 0.09*
Species richness			▲ 0.17*		▲ 0.09**				▲ 0.09*
Species shown in Fig. 2 (left):									
Arion alpinus Pollonera, 1887	X	23	▲ 1.66**		▲ 0.34*	▲ 0.52*			
Arion silvaticus Lohmander, 1937		32	△ 0.99					▲ 0.59*	▲ 0.38*
Columella aspera Waldén, 1966	X	18	▲ 1.96*						
Discus ruderatus ruderatus (W. Hartmann, 1821)	X	27	▲ 1.79**						▼ -0.43*
Euconulus fulvus (O. F. Müller, 1774)		61	▲ 0.87***		▲ 0.20*	▲ 0.35***		△ 0.24	
Limax cinereoniger Wolf, 1803		94	△ 0.34	▲ 0.16*	▽ -0.13	▲ 0.13*			
Malacolimax tenellus (O. F. Müller, 1774)		79	▲ 1.04**	▲ 0.18***	▼ -0.35***	▲ 0.29***			
Punctum pygmaeum (Draparnaud, 1801)		24	△ 0.69	△ 0.33	▲ 0.36*	▲ 0.53**	▼ -0.51*		

(continues)

(continued)

Dependant variable	Red data book	Frequency (occupied plots)	Temperature	pH-value of humus layer	Soil water balance index	Age	Coarse woody debris	Opening of canopy layer	Plant Diversity
Semilimax semilimax (J. Férussac, 1802)		28	▲ 1.06*			▲ 0.31*		▽ -0.38	
Semilimax kotulae (Westerlund, 1883)	X	72	▼ -0.70*						
Species shown in Fig. 2 (right):									
Aegopinella pura (Alder, 1830)		14							
Arion brunneus Lehmann, 1862	X	31			▲ 0.33*		△ 0.34		
Arion rufus (Linnaeus, 1758)	X	10							
Arion subfuscus (Draparnaud, 1805)		112		△ 0.08	△ -0.08	▲ 0.15**			
Cochlodina laminata (Montagu, 1803)		20				▲ 0.52*	▽ -0.72	▼ -1.15*	
Discus rotundatus (O. F. Müller, 1774)		30		▲ 0.59**		▲ 0.64***	▼ -0.55*	△ 0.37	
Eucobresia diaphana (Draparnaud, 1805)		13			▲ 0.81**				
Lehmannia marginata (O. F. Müller, 1774)		90				▲ 0.23***		▼ -0.39**	
Monachoides incarnatus (O. F. Müller, 1774)		16							△ 0.67
Nesovitrea hammonis (Ström, 1765)		40		▽ -0.32		▲ 0.51**			▲ 0.43*
Sum of significance (almost significance)			10 (3)	3 (4)	8 (2)	13	2 (2)	3 (3)	5 (1)

the number of species, species richness and abundance of single species, we used Poisson errors with a log link function and for the total number of individuals of all molluscs we used a Gaussian error with an identity link (Quinn & Keough, 2002; Everitt & Hothorn, 2006). To allow a comparison of the estimators within one model, all predictors were standardised to zero mean-unit variance. To account for spatial autocorrelation we used a semiparametric spatial generalised linear model (Dormann et al., 2007). Thereby spatial autocorrelation is alleviated by including a spatial surface in the regression model. Assuming asymptotic normality of the estimated regression coefficients, confidence bands and p-values can be computed based on the standard deviations obtained from the expected Fisher information matrix. For these calculations we used "BayesX" (Fahrmeir et al., 2004; Kneib & Fahrmeir, 2006; Kneib et al., 2008).

To estimate the influence of climate change on the altitudinal distribution of the montane species *S. kotulae*, we used logistic and Poisson regression as implemented in "BayesX" to predict the occurrence as well as abundance of this species considering all available predictors (Table 1). This analysis showed that only temperature had a significant influence on occurrence and abundance (Table 2).To predict occurrence or density of *S. kotulae* from the temperature of a site, GLMs were fitted to the occurrence (logistic model) and abundance (Poisson model) using current mean annual temperature and excluding spatial effects (see above, inset Fig. 4). Subsequently, we used these models to predict the probability of occurrence and abundance of *S. kotulae* for each plot. In a final step, we increased for each plot the mean annual temperature by the values estimated in two scenarios of global warming (IPCC, 2007a) which predict an increase in the mean annual temperature until 2100 by 1.8°K (optimistic assumptions) and by 4.0°K (pessimistic assumptions). The optimistic global estimate of warming corresponds with local models (Spekat et al., 2007). Local models are not available for the pessimistic scenario. From these temperatures, we predicted the occurrence and abundance of *S. kotulae* across the sampled plots. Finally, predictions based on the temperature based model were plotted against altitude of each plot. We visualized the general trend using a spline fit as a local smoother. We also constructed heuristic confidence bands using pointwise 95% prediction intervals calculated from the standard error of the predictions. The upper and lower limits were again estimated with smoothing splines (the R-script of this method is available by JM). Our predictions rest on the assumption that the temperature increase is the same across the altitudinal range. The analysis of temperature data from 1948 to 2002 by Bässler (2008) showed that this assumption is valid for the study region: he found a temperature increase of 0.01°K year^{-1} at all elevations.

RESULTS

In total we sampled 3,437 individuals of 46 species (Appendix). On single plots individuals ranged from 7 to 96 individuals representing 3 to 27 species. In the analysis of community characteristics as well as abundances of the more common species (at least ten occupied plots) *versus* environmental variables, the number of species and individuals, as well as the abundance of 11 species increased with tree age (Table 2). Temperature ranked second, when counting the number of models with a significant temperature effect (Table 2). Seven of the twenty single species models showed a significant response to temperature (Fig. 2 right, Table 2). Most of these responses were positive, only *Semilimax kotulae* showed a negative response (Fig. 2 right, Fig. 3a, b, Table 2).

Although species richness and abundance of molluscs (excluding *S. kotulae*) decreased with altitude and increased with temperature (Fig. 3c–f), these relationships were non-linear. Similarily, for *S. kotulae* the relationship between abundance and temperature with altitude was also non-linear (Fig. 3a, b). The quantile regression showed always change in the slopes at altitudes between 1,100 m and 1,200 m.

For obvious reasons, temperature decreased with altitude across the sampled plots, however with a number of outliers (Fig. 4). These outliers represent sinks for cold air, at the foot of the mountains. Occurrences of *S. kotulae* at lower altitudes were partly located in those sinks; other records at low altitudes were only from moorland site with cold microhabitats.

As expected, the probability of occurrence of *S. kotulae* decreased with increasing temperature (spatial GLM; $p = 0.03$; for all other variables $p > 0.25$; non spatial glm temperature ($p < 0.001$). The cross-correlogram of the residuals of the model including only the variable temperature indicated that the spatial effect can be ignored for this species (Fig. 4 inset). The predictive model indicated that an increase

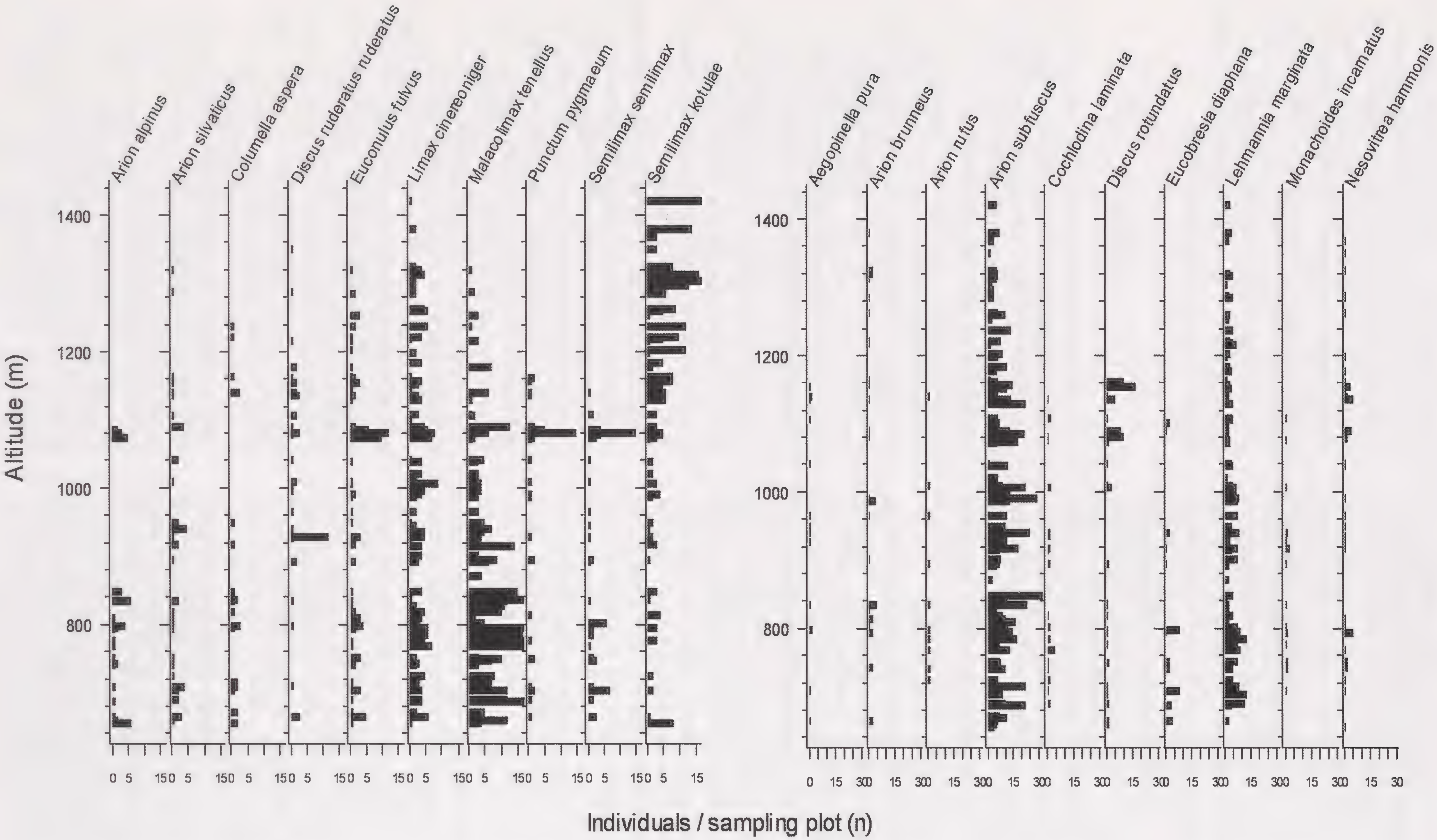

FIG. 2. Abundance of the species, occurring in at least ten sampling plots along altitudinal gradients within the Bavarian Forest National Park (each bar represents one of the 111 plots; see Fig. 1). The upper part shows species where we found at least a marginal significant relationship in spatial glm between abundance and temperature (p < 0.1) Otherwise, species are presented in alphabetical order (see also Table 2).

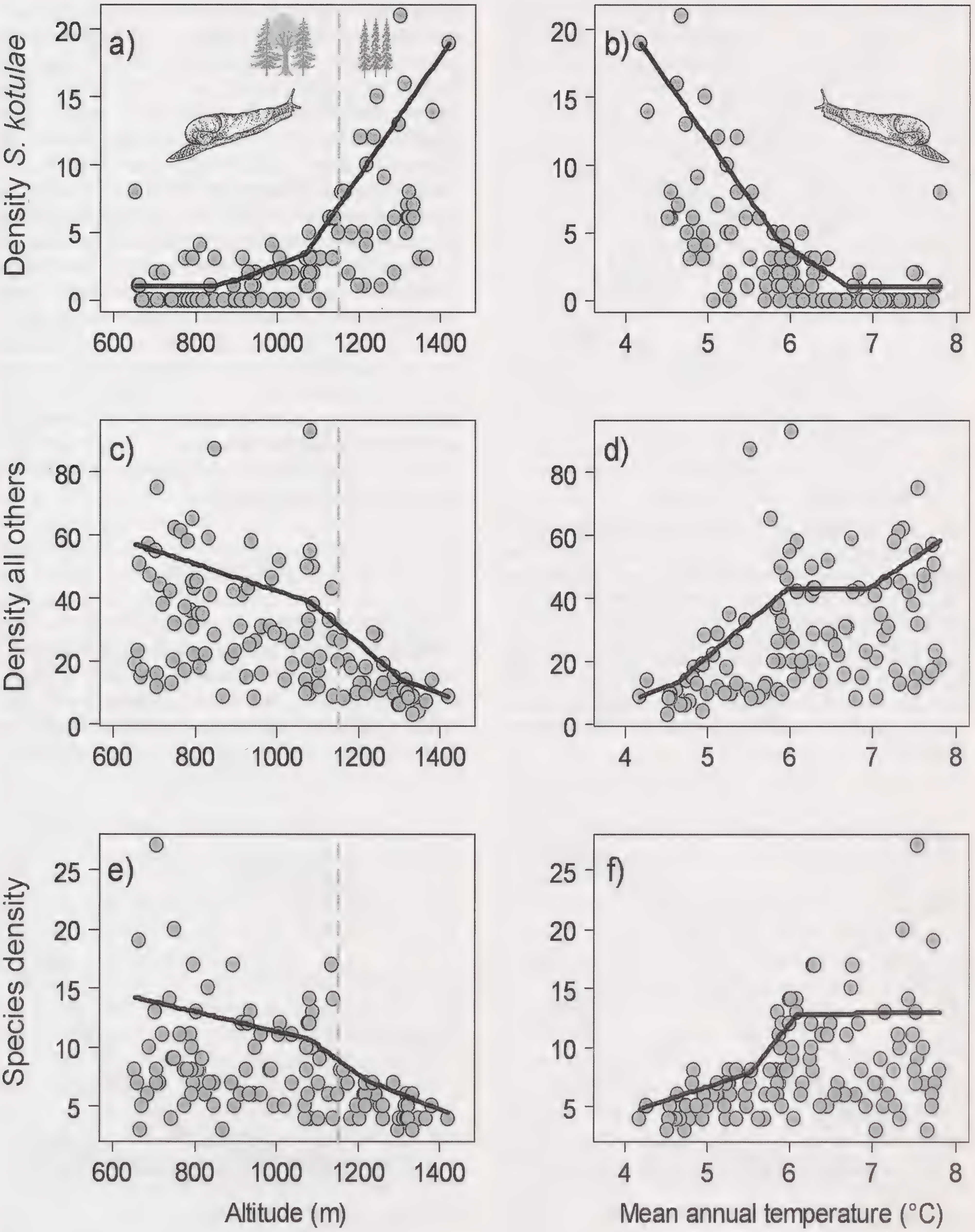

FIG. 3. Number of species, abundance of *Semilimax kotulae* as well as abundance for all species (with exception *S. kotulae*) versus altitude and temperature across 111 plots sampled within the Bavarian Forest National Park (Fig. 1). Lines are additive quantile regressions. The dashed line and the tree symbols indicate the altitude of the change between mixed montane (< 1,150 m) and high montane spruce forests (> 1,150 m).

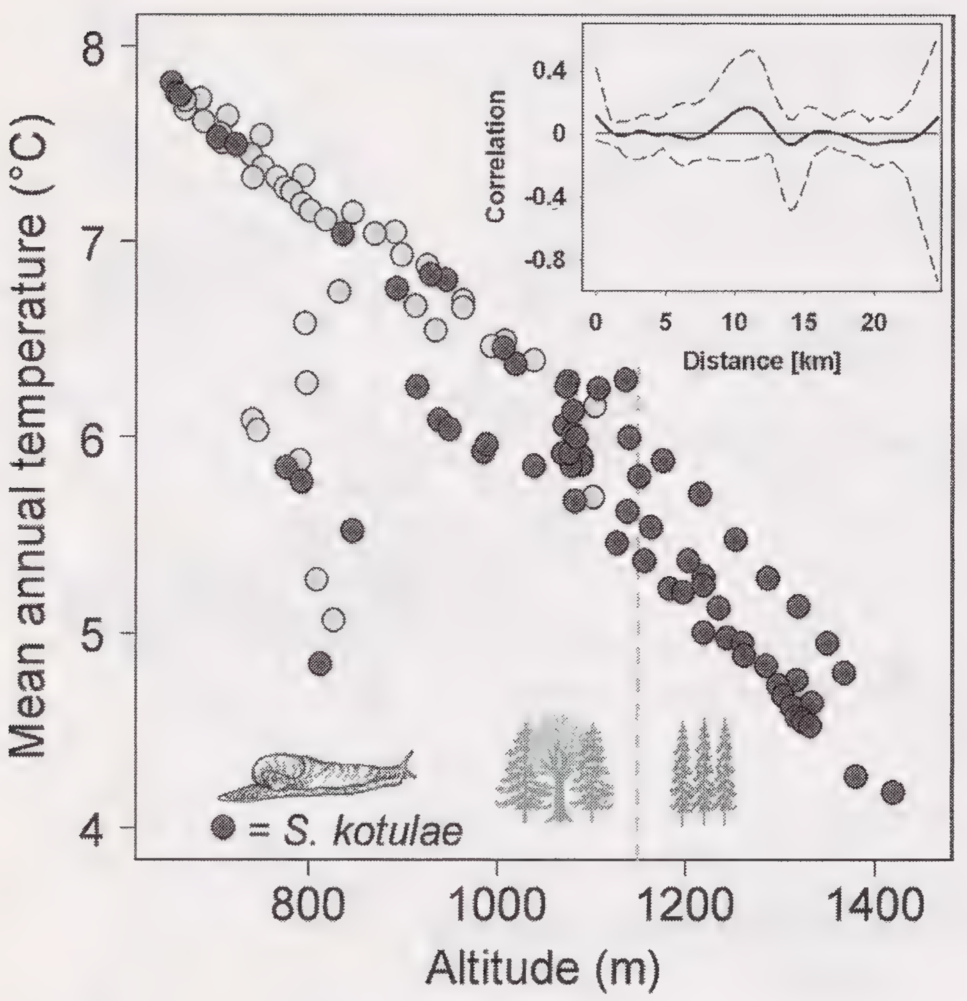

FIG. 4. Relation of mean annual temperature and altitude across the 111 sampling plots in the Bavarian Forest National Park. The outliers are typical sinks for cold air. Dark symbols indicate plots where we recorded *Semilimax kotulae*. Inset shows the spatial autocorrelation of the residuals of a glm with presence/absence data for *S. kotulae* and temperature as predictor. The dashed line indicates the altitude of the change between mixed montane and high montane spruce forests (1,150 m).

in the mean annual temperature of 1.8°K will already lead to a decrease of occurrences by about 50% at altitudes of 1,300 m (Fig. 5). Note that most mountains in the region are below this altitude. Nevertheless, within this optimistic scenario some sinks for cold air at lower altitudes may act as refuges for *S. kotulae* assuming that local circulation patterns of the air will not change with global warming. An increase in mean annual temperature of 4.0°K would probably lead to the extinction of *S. kotulae* in the national park. Modelling abundance produced even more pessimistic results (Fig. 5). Again taking 1,300 m as an example the model predicts abundances of ≈1 if mean annual temperature increases by 1.8°K and close to 0 for an increase of 4.0°K. Note that at present abundances are ≈ 7. These predictions do not change considering temperature and precipitation together.

DISCUSSION

Even though the decrease of diversity along altitudinal gradients has fascinated biologists since Darwin, the reasons are still unclear (Gaston, 2000). We found a non-linear decrease of species richness in molluscs. Aubry et al. (2005) studied richness of molluscs in

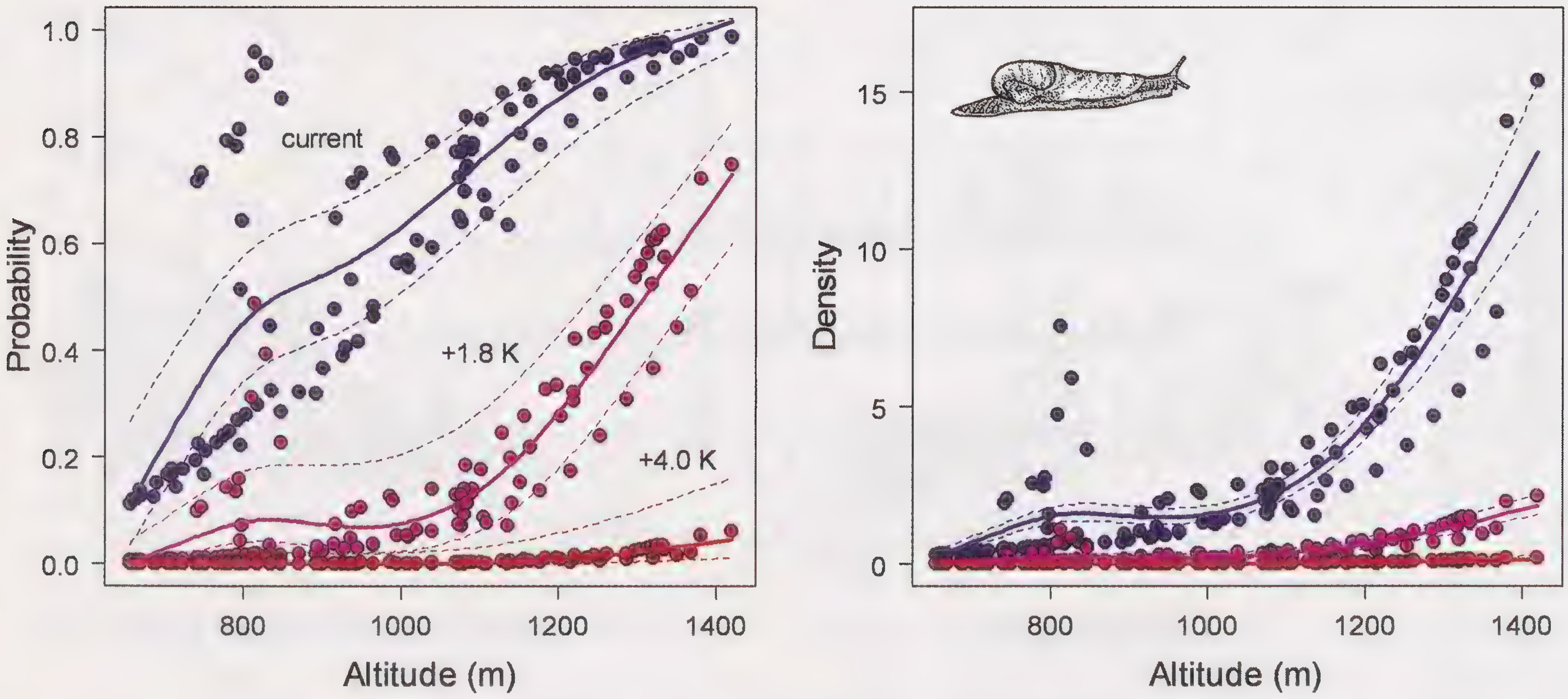

FIG. 5. The blue symbols indicate the predicted probability of occurrences (left) and abundance (right) of *Semilimax kotulae* across the 111 sampling plots versus altitude within the National Park "Bavarian Forest" using annual mean temperature. The blue curves are a local smoother (spline) with heuristic confidence bands (for details see Material and Methods). The other two groups of points and curves are predictions for *S. kotulae* using scenarios of global warming with an increase of the mean annual temperature by 1.8°K and 4.0°K.

calcareous mountains in southeastern France, where they found a plateau of species richness at elevations between 200 m and 1,000 m. Below and above this range they documented a decrease in species richness. Even though this study was undertaken in an area geologically and climatically very much different to our study area, the diversity patterns in our area are rather similar. We found a change in the slope of the decrease of species richness with altitude at around 1,150 m, the altitude where the switch from mixed montane to high montane forests occurs. Several studies have already stressed the close correlation between altitudinal zonation of molluscs and plant communities (e.g., Coppois, 1984).

Körner (2007) noted the difficulty of interpreting effects of non-climatic variables that are strongly correlated with elevation only within certain regions. Such variables can modify altitudinal patterns of diversity in such a way that climate seems to be unimportant (Lee et al., 2004). Nevertheless, Aubry et al. (2005) suggest that climate and the availability of microhabitats determines the number of species. Clearly, our results are consistent with this idea. Temperature, soil water balance, age of trees and plant species diversity are significantly and positively correlated with the densities of several species (Barker & Mayhill, 1999; Müller et al., 2005a; Horsák, 2006). Habitat suitability should be even of more importance in our study area than in the area studied by Aubrey et al. (2005). The sampled region is characterized by acidic soils whereas molluscs often prefer habitats with high pH values (Wäreborn, 1969; Waldén, 1981; Coney et al., 1982; Martin & Sommer, 2004; Horsák, 2006).

Tree age was of overwhelming importance in our models. Furthermore, abundance and number of species also increased with tree age. In forests, the age of the oldest tree in a stand is a surrogate of habitat continuity (Speight, 1989; Sverdrup-Thygeson & Lindenmeyer, 2003; Müller et al., 2005b). Continuity is an important factor for the distribution of molluscs on a regional scale. For example, the abundance of *Limax cinereoniger* and *Macrogastra plicatula plicatula* increases with stand age and therefore habitat continuity (Müller et al., 2005a; Bußler et al., 2007). The importance of continuity for molluscs has at least three components: Firstly, some species feed on the bark of old broadleaved trees and therefore require veterans. Such species are *Clausilia cruciata cruciata* or *Lehmannia marginata*, which at least in Bavaria occurs only in forests (Falkner, 1991; Hässlein, 1966). Secondly, several species need dead wood to obtain calcium, for example *Macrogastra badia crispulata*, *M. plicatula nana*, *Causa holosericea*, *Discus ruderatus ruderatus* (Kappes, 2005; Strätz & Müller, 2006). Thirdly, some mollusc species are consumers of wood-inhabiting or hypogeous fungi (*Arion alpinus*, *Malacolimax tenellus*), which are more common in old stands (Junninen & Angelstam, 2006). Old forests are not only important to molluscs with their low propensity for dispersal (Ant, 1963; Shikov, 1984; Rüetschi, 1999; Strätz, 2005), but also for lichens, wood-inhabiting fungi, saproxylic beetles, or birds (Stubbs, 1989; Bader et al., 1995; Nilsson et al., 1995; Ulikzka et al., 2000; Gustafsson et al., 2004). Our results once more underline the importance of strict forest reserves for conserving the biodiversity of a wide variety of organisms (Christensen et al., 2005).

Overall, we found an increase in number of species and species richness with temperature. This suggests that climate warming will lead to an increase of species richness of molluscs in the national park for two reasons (Grabherr et al., 1994). Firstly, low altitude species will be able to shift the upper distributional limits to higher altitudes. Secondly, new species will be able to invade the area (including such alien species as *Arion lusitanicus*). Therefore, viewed naively, climate warming will be a positive phenomenon if one considers only species richness. However, for species adapted to cool, montane habitats climate warming may lead to local or regional extinction, especially because the available area suitable to them is limited at low mountain ranges. Our example, *Semilimax kotulae*, quantifies this dramatic risk of climate warming.

Semilimax kotulae is endemic to the Alpine-Carpathian area and occurs at altitudes above 600 m. Populations between 500 and 600 m exist in sites where cold air emanates from blocky scree slopes (Hässlein, 1966), which is fully in line with our results. When we compared our data with data from whole Bavaria (Strätz, unpublished) or the East Carpathians (Sulikowska-Drozd & Horsák, 2007), we found a very similar pattern of altitudinal distribution of *S. kotulae*, which suggests that this species may be a suitable indicator for the fate of montane species affected by global warming in whole Central Europe (see also De Groot et al., 1995; Kappelle et al., 1999). As indicated by our exercise presented in Figure 5, an increase in the mean annual temperature of more than 1.8°K will increase the risk of extinction for this species and, if one accepts that *S. kotulae* may be used as an indicator species, of other montane species. Under pessimistic scenarios of climate warming, *S. kotulae* will only survive in regions that provide sufficient habitats at higher altitudes (> 1,300 m; Alps). Note also that the cool plots which at present still harbour populations of *S. kotulae* will also loose their suitability for this species (Fig. 5). The negative response of *S. kotulae* to higher temperatures may have two reasons: for eggs and juveniles a constant humidity is necessary. Humidity decreases with increasing temperature (Uminski, 1975). A second reason may be competition. Even though the biology of *S. kotulae* is still not well known, this species is described as a weak competitor (Falkner, 1991). At lower elevations, four species with similar habitat requirements occur (*Vitrina pellucida*, *Semilimax semilimax*, *Eucobresia diaphana* and *Vitrionobrachium breve*, Falkner, 1991) which may outcompete *S. kotulae.*

During recent years studies have demonstrated that projections produced by alternative models are sometimes such variable that their usefulness for policy decisions is compromised (Araújo et al., 2006). For example, different models predict changes in the distribution from 92% loss to a 323% gain for a South African plant species (Pearson et al., 2006). Such problems call for the application of a combination of modelling techniques (Drake et al., 2006). We checked our results by including additionally precipitation into the model. However, this model produced very similar results. Furthermore, the very similar conclusions from models using presence/absence and abundance data suggest that our conclusion is fairly robust: *S. kotulae* may disappear in the near future from the national park (cf. Berg et al., 2004).

The upper altitudinal zones of the low mountain ranges in Central Europe are not species rich when compared to lowland forests. Nevertheless, several species are restricted to this zone. Most of them are recorded only once or a few times during the last decades (e.g., *Vertigo modesta arctica*, *Vertigo alpestris*), and therefore they are not suited as indicators. Nevertheless their present range of occurrence is between 1,100 m and 1,400 m a.s.l., and these species are faced with a similar risk as *S. kotulae*. Overall global warming constraints one of the major aims of the Bavarian Forest National Park: the conservation of species assemblages of the high montane zone.

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APPENDIX

Species *versus* plot matrix. Numbers indicate individuals; Temp gives the modelled temperature for each plot based on geostatistical modelling see Methods. Note, that two species of genus *Pisidium* which were sampled in a few small ephemeral water bodies within forest stands were also included.

Plot	Temp (in °C)	NN (in m)	*Acanthinula aculeata*	*Aegopinella nitens*	*Aegopinella pura*	*Arianta arbustorum*	*Arion alpinus*	*Arion brunneus*	*Arion distinctus*	*Arion lusitanicus*	*Arion rufus*	*Arion silvaticus*	*Arion subfuscus*	*Balea perversa*	*Boettgerilla pallens*	*Carychium tridentatum*	*Causa holosericea*	*Clausilia cruciata cruciata*	*Cochlicopa lubrica*	*Cochlodina laminata*	*Columella aspera*	*Columella edentula*	*Deroceras laeve*	*Deroceras reticulatum*	*Discus rotundatus*	*Discus ruderatus ruderatus*	*Eucobresia diaphana*	*Euconulus fulvus*	*Isognomostoma isognomostomos*	*Lehmannia marginata*	*Limax cinereoniger*	*Macrogastra plicatula nana*	*Malacolimax tenellus*	*Monachoides incarnatus*	*Nesovitrea hammonis*	*Nesovitrea petronella*	*Oxychilus cellarius*	*Petasina edentula liminifera*	*Pisidium casertanum*	*Pisidium personatum*	*Punctum pygmaeum*	*Radix labiata*	*Semilimax kotulae*	*Semilimax semilimax*	*Vertigo pusilla*	*Vertigo substriata*	*Vitrea subrimata*	*Vitrina pellucida*
T1_27	6.27	798.08			2		4	1			1		9			1					1				1	1	8	4		2	1		2		1				2					2				
T1_3	6.08	741.62					2	2	1	3			7		1	3							2	1				2							2		1		5			10						
T1_30	6.74	833.67			1		6	5			1	2	23								1				1	1		1		2			12	1		1								1				
T1_39	6.58	796.92										1	7								3				1						3		5											2				
T1_4	6.03	746.10					1						4			1														1	1		7	1	1									3				
T1_49	7.05	891.47											5													2		2		2	3		6											1				
T1_51	6.87	926.06			1								4													1		1		2	1		4											1				
T1_52	6.80	945.35											1																	1	2		3										1	1				
T1_53	6.69	964.28			1			1			1		11			2												1		7	2		3		1									1				
T1_55	6.48	1009.63									1	1	9			1									1	2		1		5	2		3								1			1				
T1_56	6.39	1040.28			1							2	1												1	1				4	1		5		1						1			1				
T1_58	6.15	1104.93			1							1	5													2				5	1		2															
T1_59	5.99	1140.64			2						1	1	6			1									1	2		1		2			6		2						1		3	1				
T1_60	5.87	1176.55											5													2		1		4			7		1								2					
T1_61	5.70	1215.29											2													1		1		6			3										1					
T1_62	5.47	1253.49											3															3		2			3										1					
T1_63	5.27	1287.09										1	3													1				2			2		1								2					

(continues)

(continued)

Plot	Temp (in °C)	NN (in m)	*Acanthinula aculeata*	*Aegopinella nitens*	*Aegopinella pura*	*Arianta arbustorum*	*Arion alpinus*	*Arion brunneus*	*Arion distinctus*	*Arion lusitanicus*	*Arion rufus*	*Arion silvaticus*	*Arion subfuscus*	*Balea perversa*	*Boettgerilla pallens*	*Carychium tridentatum*	*Causa holosericea*	*Clausilia cruciata cruciata*	*Cochlicopa lubrica*	*Cochlodina laminata*	*Columella aspera*	*Columella edentula*	*Deroceras laeve*	*Deroceras reticulatum*	*Discus rotundatus*	*Discus ruderatus ruderatus*	*Eucobresia diaphana*	*Euconulus fulvus*	*Isognomostoma isognomostomos*	*Lehmannia marginata*	*Limax cinereoniger*	*Macrogastra plicatula nana*	*Malacolimax tenellus*	*Monachoides incarnatus*	*Nesovitrea hammonis*	*Nesovitrea petronella*	*Oxychilus cellarius*	*Petasina edentula liminifera*	*Pisidium casertanum*	*Pisidium personatum*	*Punctum pygmaeum*	*Radix labiata*	*Semilimax kotulae*	*Semilimax semilimax*	*Vertigo pusilla*	*Vertigo substriata*	*Vitrea subrimata*	*Vitrina pellucida*
T1_64	5.13	1319.59										1	6															1		3			1										7					
T1_65	4.94	1350.04											2													1									1								3					
T1_66	4.78	1367.99											4																	2					1								3					
T1_9	5.87	791.70					1	1				1	11												1			2			1		9	2	5									2				
T2_2	5.84	777.69					1						5												1					7			21	1							1		3					
T2_23	5.06	827.25						1					5															1		2	2		11															
T2_25	4.84	813.40						2					10															1			1		2		1						1		4					
T2_27	5.27	809.53					1					1	16															3		2	4		2						6									
T2_3	5.77	794.09					2			1			15															1		9	5		32										3					
T2_35	6.26	916.17	1									2	18							2	1						1	2		7			3	3	1								3					
T2_36	6.09	938.89			1	2						5	25					1		2						1	2			8	2		7	2									1					
T2_38	6.03	949.31			1							2	10								1							1		4	1		5		1								2					
T2_41	5.92	986.54						4					13															1		7	2		3								1		2					
T2_44	5.96	990.01						1					29															2		8	4				1						1		4					
T2_48	5.85	1039.44											12															1		5	4		3		1								2					
T2_50	5.67	1082.01											6																		1		3										2					
T2_52	5.45	1128.13											22															1		5	4		1										6					
T2_53	5.36	1157.20						1					15															1		3	4				1						1		8					
T2_54	5.22	1183.69											11																	3	4												5					
T2_67	4.18	1419.52											5																	3	1												19					
T2_69	4.26	1380.01						1					7																	4	2												14					

(continues)

(continued)

Plot	Temp (in °C)	NN (in m)	*Acanthinula aculeata*	*Aegopinella nitens*	*Aegopinella pura*	*Arianta arbustorum*	*Arion alpinus*	*Arion brunneus*	*Arion distinctus*	*Arion lusitanicus*	*Arion rufus*	*Arion silvaticus*	*Arion subfuscus*	*Balea perversa*	*Boettgerilla pallens*	*Carychium tridentatum*	*Causa holosericea*	*Clausilia cruciata cruciata*	*Cochlicopa lubrica*	*Cochlodina laminata*	*Columella aspera*	*Columella edentula*	*Deroceras laeve*	*Deroceras reticulatum*	*Discus rotundatus*	*Discus ruderatus ruderatus*	*Eucobresia diaphana*	*Euconulus fulvus*	*Isognomostoma isognomostomos*	*Lehmannia marginata*	*Limax cinereoniger*	*Macrogastra plicatula nana*	*Malacolimax tenellus*	*Monachoides incarnatus*	*Nesovitrea hammonis*	*Nesovitrea petronella*	*Oxychilus cellarius*	*Petasina edentula liminifera*	*Pisidium casertanum*	*Pisidium personatum*	*Punctum pygmaeum*	*Radix labiata*	*Semilimax kotulae*	*Semilimax semilimax*	*Vertigo pusilla*	*Vertigo substriata*	*Vitrea subrimata*	*Vitrina pellucida*
T2_73	4.76	1317.97						1					6																	5	2												5					
T2_75	4.83	1285.57						1					4															2		5	2				1								6					
T2_79	5.29	1218.65						1					2															1		5	1												5					
T2_81	5.36	1202.83											9															1		3													12					
T2_9	5.52	847.50					3						60															1		4	4		15										3					
T3_1	7.81	655.00					6						4								2							1			1		4		1								8					
T3_12	7.64	714.98											22								2				1			1		8	3		7															
T3_17	7.54	750.19										1	8									3			2		2			3	1		10								2							
T3_22	7.33	795.42											12								1				1		1	2		5	6		15	1	1													
T3_23	7.32	812.49											7																				1										1					
T3_25	7.14	846.87											8								1									5	4		10															
T3_26	7.04	870.08											3																	2			4															
T3_27	6.93	900.20						1					8																	7	4		3															
T3_28	6.83	929.44										1	11													12		3		3	5		4	1	1						1		2	1				
T3_29	6.65	964.35											2													1		1		7	2		3															
T3_3	7.75	660.02					2					1	5												2						1		12										1					
T3_30	6.46	994.48											13													1				6	5		4															
T3_31	6.37	1020.19											6																	1	4		3										2					
T3_33	6.06	1072.47											7																		5		2										3					
T3_34	5.89	1089.60										4	13												8	1		2		2	5		13								2		3					
T3_35	5.86	1090.18						1				2	10												3	1	1	2		1	2		10		4						1		2					

(continues)

(continued)

Plot	Temp (in °C)	NN (in m)	*Acanthinula aculeata*	*Aegopinella nitens*	*Aegopinella pura*	*Arianta arbustorum*	*Arion alpinus*	*Arion brunneus*	*Arion distinctus*	*Arion lusitanicus*	*Arion rufus*	*Arion silvaticus*	*Arion subfuscus*	*Balea perversa*	*Boettgerilla pallens*	*Carychium tridentatum*	*Causa holosericea*	*Clausilia cruciata cruciata*	*Cochlicopa lubrica*	*Cochlodina laminata*	*Columella aspera*	*Columella edentula*	*Deroceras laeve*	*Deroceras reticulatum*	*Discus rotundatus*	*Discus ruderatus ruderatus*	*Eucobresia diaphana*	*Euconulus fulvus*	*Isognomostoma isognomostomos*	*Lehmannia marginata*	*Limax cinereoniger*	*Macrogastra plicatula nana*	*Malacolimax tenellus*	*Monachoides incarnatus*	*Nesovitrea hammonis*	*Nesovitrea petronella*	*Oxychilus cellarius*	*Petasina edentula liminifera*	*Pisidium casertanum*	*Pisidium personatum*	*Punctum pygmaeum*	*Radix labiata*	*Semilimax kotulae*	*Semilimax semilimax*	*Vertigo pusilla*	*Vertigo substriata*	*Vitrea subrimata*	*Vitrina pellucida*
T3_36	5.85	1079.73						1					7																	1	1		1										1					
T3_37	5.91	1069.87											5																	3			1								1		1					
T3_45	5.69	1102.02											7														2			2	1																	
T3_48	5.62	1139.15											5								3										1												6					
T3_49	5.54	1163.20						1				1	2								1							1		1	1												8					
T3_52	5.24	1219.57						1					8								1							1		3	4												10					
T3_53	5.13	1236.23											14								1							2		5	6		1										12					
T3_57	4.94	1260.55											10																	3	6												5					
T3_9	7.73	686.59					1						22														3			4	4		23															
T4_14	7.67	668.72								1			11																				3															
T4_20	7.53	708.49			1								5					2			2									1	2		3															
T4_21	7.50	709.21		1	1								2					2		1						1				1			3															
T4_22	7.53	703.01										2	9					1			1				1			3	1	12	4		12								2		2	7				
T4_24	7.61	689.91										2	10							2					2			1		11	4		12								1			2				
T4_28	7.75	663.87			1	1	1	2				3	6									2			2	3	4	5		2	6		3					1	5		1		1	3				
T4_29	7.72	669.94											7								2							1			2		5															
T4_35	7.54	707.10					1			13		4	6		1	3		1		1		8		1			8	1		2	1	1	2	1	1	5		2	3		1			2	2	2	1	1
T4_36	7.50	722.71								2	1	1	6					7		2										5	5		8		1								2					
T4_37	7.44	739.53					1			3	1	1	10					5		1		4					2	2		5	2		3	2														
T4_38	7.37	751.68				1	1			7		1	7					3		1		4					2	3		7	2		3	2	2	1		1	10	2				2				
T4_39	7.32	766.85					1			2	1		13							4								1		9	7		21		1									1				

(continues)

(continued)

Plot	Temp (in °C)	NN (in m)	*Acanthinula aculeata*	*Aegopinella nitens*	*Aegopinella pura*	*Arianta arbustorum*	*Arion alpinus*	*Arion brunneus*	*Arion distinctus*	*Arion lusitanicus*	*Arion rufus*	*Arion silvaticus*	*Arion subfuscus*	*Balea perversa*	*Boettgerilla pallens*	*Carychium tridentatum*	*Causa holosericea*	*Clausilia cruciata cruciata*	*Cochlicopa lubrica*	*Cochlodina laminata*	*Columella aspera*	*Columella edentula*	*Deroceras laeve*	*Deroceras reticulatum*	*Discus rotundatus*	*Discus ruderatus ruderatus*	*Eucobresia diaphana*	*Euconulus fulvus*	*Isognomostoma isognomostomos*	*Lehmannia marginata*	*Limax cinereoniger*	*Macrogastra plicatula nana*	*Malacolimax tenellus*	*Monachoides incarnatus*	*Nesovitrea hammonis*	*Nesovitrea petronella*	*Oxychilus cellarius*	*Petasina edentula liminifera*	*Pisidium casertanum*	*Pisidium personatum*	*Punctum pygmaeum*	*Radix labiata*	*Semilimax kotulae*	*Semilimax semilimax*	*Vertigo pusilla*	*Vertigo substriata*	*Vitrea subrimata*	*Vitrina pellucida*
T4_4	7.27	774.83											7															1		3	5		1															
T4_40	7.25	782.44								1	1		17							2										12	6		17											2				
T4_41	7.19	793.14						2					12																	7	4		5											1				
T4_42	7.15	803.04						1				1	12							2		1						1		7	3		5	1	1				4					6				
T4_43	7.10	817.95										1	9							1	1				1			2		5	5		10															
T4_44	7.03	836.09											11								2				1					1	1		25										1					
T4_47	6.75	893.93									1	1	7				4			2		4			2		1			2	1		9	1							2		1	2		2	1	
T4_48	6.67	914.29											7				1			1										4	4		14															
T4_49	6.54	936.08											10							2										2	5		5		1													
T4_51	6.45	1006.87				2							22							3					3	1		1		6	9		4	1									3					
T4_53	6.28	1076.29											15							1					2					3	5		2	1									2					
T4_54	6.24	1108.10											6							3					1					4	3		1	1									3	2				
T4_55	6.29	1135.87			1			1				1	14						1	1					5	3		2		1	3				5						1		3			1	2	1
T4_56	6.17	1153.76			1							1	2												17	2		3			2				3								1				1	
T4_57	6.04	1161.32										1	6	1					1						10	1		2							1						2		3				1	1
T4_59	5.80	1150.50											11															1		4	3				1								5					
T4_7	7.32	742.03											5															1		4	3																	
T4_70	5.21	1197.20											6																	1	2				1								1					
T4_72	4.99	1219.93						1					7																	1	1												4					
T4_76	4.88	1261.64						1					6																		5				1								9					
T4_78	4.72	1297.51											4																		2												13					

(continues)

(continued)

Plot	Temp (in °C)	NN (in m)	*Acanthinula aculeata*	*Aegopinella nitens*	*Aegopinella pura*	*Arianta arbustorum*	*Arion alpinus*	*Arion brunneus*	*Arion distinctus*	*Arion lusitanicus*	*Arion rufus*	*Arion silvaticus*	*Arion subfuscus*	*Balea perversa*	*Boettgerilla pallens*	*Carychium tridentatum*	*Causa holosericea*	*Clausilia cruciata cruciata*	*Cochlicopa lubrica*	*Cochlodina laminata*	*Columella aspera*	*Columella edentula*	*Deroceras laeve*	*Deroceras reticulatum*	*Discus rotundatus*	*Discus ruderatus ruderatus*	*Eucobresia diaphana*	*Euconulus fulvus*	*Isognomostoma isognomostomos*	*Lehmannia marginata*	*Limax cinereoniger*	*Macrogastra plicatula nana*	*Malacolimax tenellus*	*Monachoides incarnatus*	*Nesovitrea hammonis*	*Nesovitrea petronella*	*Oxychilus cellarius*	*Petasina edentula liminifera*	*Pisidium casertanum*	*Pisidium personatum*	*Punctum pygmaeum*	*Radix labiata*	*Semilimax kotulae*	*Semilimax semilimax*	*Vertigo pusilla*	*Vertigo substriata*	*Vitrea subrimata*	*Vitrina pellucida*
T4_79	4.67	1303.55											3																	1	2												21					
T4_80	4.62	1312.11						1					5																		5												16					
T4_81	4.56	1318.54						2					4																		4												6					
T4_82	4.56	1322.92						2					4																		1				1								8					
T4_83	4.54	1325.09						1					5																		2												6					
TS_1	6.13	1080.25					1						10															3			5		1										5					
TS_2	5.91	1076.80					3						9					1							1	1		5			4		2		2						1		5	4				
TS_3	6.01	1081.29					3						16							1					10	3		12		2	8	1	6		1						15		3	15				
TS_4	5.99	1083.80					2						21												3	1		6		2	6		4		1						5		2	4				
TS_5	6.25	1073.47					5						18												2			10		1	7		2								2		1	3				

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KARYOTYPES, BANDING PATTERNS AND NUCLEAR DNA CONTENT IN *CREPIDULA UNGUIFORMIS* LAMARCK, 1822, AND *NATICARIUS STERCUSMUSCARUM* (GMELIN, 1791) (MOLLUSCA, CAENOGASTROPODA)

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ABSTRACT

The chromosome complement and the nuclear DNA content in two caenogastropod species from the Mediterranean Sea, *Crepidula unguiformis* (Calyptraeidae) and *Naticarius stercusmuscarum* (Naticidae), were investigated by the application of both classical and molecular cytogenetic methods. Despite the constancy of haploid chromosome numbers (n = 17 in both species), *C. unguiformis* and *N. stercusmuscarum* show genome sizes amounting to 6.36 and 2.63 pg, respectively. Moreover, while *N. stercusmuscarum* resembles cytogenetically the other neotaenioglossan caenogastropods studied so far, *C. unguiformis* differs in: (i) number and location of rDNA clusters (ii), composition of telomeric repeats, and (iii) genome size, thus suggesting a peculiar karyotypical evolution undergone by this species.

Keywords: chromosomes, banding techniques, FISH, telomeres, genome size, Mollusca, Caenogastropoda.

INTRODUCTION

Cytogenetic information available in the literature on the molluscan superorder Caenogastropoda reports chromosome number, karyotypic macrostructure, chromosomal location of active nucleolus organizer regions (NORs) by silver staining, and/or genome size for approximately 130 species belonging to 20 families (Patterson, 1969; Thiriot-Quievreux, 2003; Gregory, 2008). Nearly nothing or very little is known about many caenogastropod families, including Calyptraeidae and Naticidae.

In the present paper we set out to investigate *Crepidula unguiformis* Lamarck, 1822 (Calyptraeidae), and *Naticarius stercusmuscarum* (Gmelin, 1791) (Naticidae) with the aim of contributing to fill the gap in cytogenetical knowledge of Caenogastropoda. For both species, the haploid chromosome number (n = 17) was the only datum available from the literature (Vitturi et al., 1982; Vitturi & Catalano, 1984). Chromosome analysis has been carried out using both classical (Giemsa-, silver- and fluorochrome staining, C-banding) and molecular [rDNA- and $(TTAGGG)_n$ FISH] methods. Moreover, the genome size was determined for both species. Obtained results are compared to cytological data reported for other Caenogastropoda.

MATERIALS AND METHODS

Animals

Specimens of the two species were collected from the coast around Palermo (northwestern Sicily, Italy), Venice (northeastern Italy), and Fano (Marche Region, Central East Italy) in 2003, 2004, 2006 and 2007. *Crepidula unguiformis* is a protandric hermaphrodite, whereas *N. stercusmuscarum* is gonochoristic. Sexually mature individuals occurred from December to April (*C. unguiformis*) and from November to January (*N. stercusmuscarum*) and were collected only in Sicily. *Crepidula unguiformis* was typically found inside dead shells of *Astraea rugosa* (Linnaeus, 1758) and *Hexaplex trunculus* (Linnaeus, 1758).

Chromosome Preparations and Banding Methods

Spermatocytal and spermatogonial chromosome plates were obtained from testes of sexually mature individuals kept in 0.01% colchicine sea water for 2 hours. Gonads were dissected and treated following the air-drying technique described elsewhere (Vitturi, 1992).

Chromosomes were directly stained with Giemsa to describe their number and morphology,

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or subjected to BSG C-banding (with barium hydroxide, saline and Giemsa, according to Sumner, 1990) to reveal constitutive heterochromatin, or banded with the fluorochromes DAPI (AT-specific) and CMA_3 (GC-specific) to show peculiar compartmentalisation of DNA (Sumner, 1990), or stained with silver nitrate to detect previously active nucleolar organizer regions (NORs) (Howell & Black, 1980).

Karyograms of *C. unguiformis* (eight spreads analysed) and *N. stercusmuscarum* (six spreads) were constructed from Giemsa-stained spermatogonial metaphases by pairing chromosomes according to dimensions and arm ratio. Nomenclature is according to Levan et al. (1964).

Fluorescent In Situ Hybridization (FISH)

FISH was performed on fixed chromosome plates from testis (as described by Vitturi et al., 2000) by hybridization with two different probes: the 18S rDNA partial sequence from sea urchin (*Paracentrotus lividus* Lamarck 1816) (Cantone et al., 1993) provided by R. Barbieri (University of Palermo) and $(TTAGGG)_n$ vertebrate-type telomeric hexamer repeat. The latter probe was obtained by PCR in the absence of template (Ijdo et al., 1991) using $(TTAGGG)_5$ and $(CCCTAA)_5$ as primers. The PCR product was DIG-labelled following the random priming protocol (Roche). Nick translation labelling with digoxigenin of the 18S rDNA was performed following manufacturer's instructions (Roche). Slides were mounted in antifade solution containing propidium iodide (2 μg/ml) and observed on a Leica epifluorescence microscope equipped with a I3 filter set (BP 450–490, LP 515) allowing the simultaneous visualisation of both fluorescein-labelled hybrid (yellow) and chromosomal DNA (red).

Genome Size (GS) Evaluation

GS was evaluated through flow cytometric assay performed on cell suspensions of *C. unguiformis* (eight samples analysed from individuals collected in Venice) and *N. stercusmuscarum* (23 samples from individuals collected in Fano) obtained from deep frozen digestive gland. The analysis was comparatively extended to five specimens of the neogastropod *H. trunculus* (from Venice), the GS of which had already been determined by Pascoe et al. (2004) with a different method (Feulgen densitometry). Peripheral blood erythrocytes from chicken (2C GS = 2.5 pg, Tiersch et al., 1989) were added to the molluscan cell suspension as internal standard. The methods for sample preparation and nuclear DNA content evaluation were the same described in Libertini et al. (2004). A BRITE-HS cytometer (Bio-Rad Laboratories) equipped with a xenon-mercury lamp was used for the analyses. Data were reported as mean ± standard deviation.

RESULTS AND DISCUSSION

Chromosome Number and Karyotype

In both species the same diploid number 2n = 34 was determined from counts of spermatogonial chromosomes, confirming the correspondent haploid value (n = 17) reported in previous papers (Vitturi et al., 1982; Vitturi & Catalano, 1984).

In the *C. unguiformis* karyotype (Fig. 1A) there are 11 metacentric/submetacentric and six subtelocentric (Fig. 1A – pairs 2, 5, 8, 13, 15, and 17) pairs. Chromosome length varies from 7.8 to 2.5 μm. In about one third of the observed metaphase figures, the chromosomes of pair 17 appear as heteromorphic. Such heteromorphism is likely due to different numbers of DNA units accumulated in the two homologues. In fact, the possibility that it may be linked to a sex determining mechanism is easily ruled out since hermaphrodite organisms cannot have sex linked chromosomes. Karyotype of *N. stercusmuscarum* (Fig. 1B) shows 12 metacentric/submetacentric and 5 subtelocentric/acrocentric (Fig. 1B – pairs 6, 11, 13, 16, and 17) homomorphic pairs. Chromosome length ranges from 3.5 to 1.2 μm.

BSG-C Banding, Fluorochrome Staining, rDNA FISH, and Ag-NOR Banding

BSG-C banding of spermatogonial chromosomes in *C. unguiformis* showed the occurrence of minute positive bands terminally and/or interstitially located (Fig. 2A), suggesting a low heterochromatin amount. In *N. stercusmuscarum*, dark heterochromatic blocks occupied the pericentromeric region of all chromosomes (Fig. 2B) plus the ends of the long arm in two large metacentric homologues which, according to their morphology and dimension, may be tentatively assigned to pair 3 of the karyotype (Fig. 2B, arrows; Fig. 1B).

In *C. unguiformis*, staining with DAPI did not reveal any brighter areas (Fig. 3A), whereas with CMA_3 (Fig. 4A) small positive interstitial

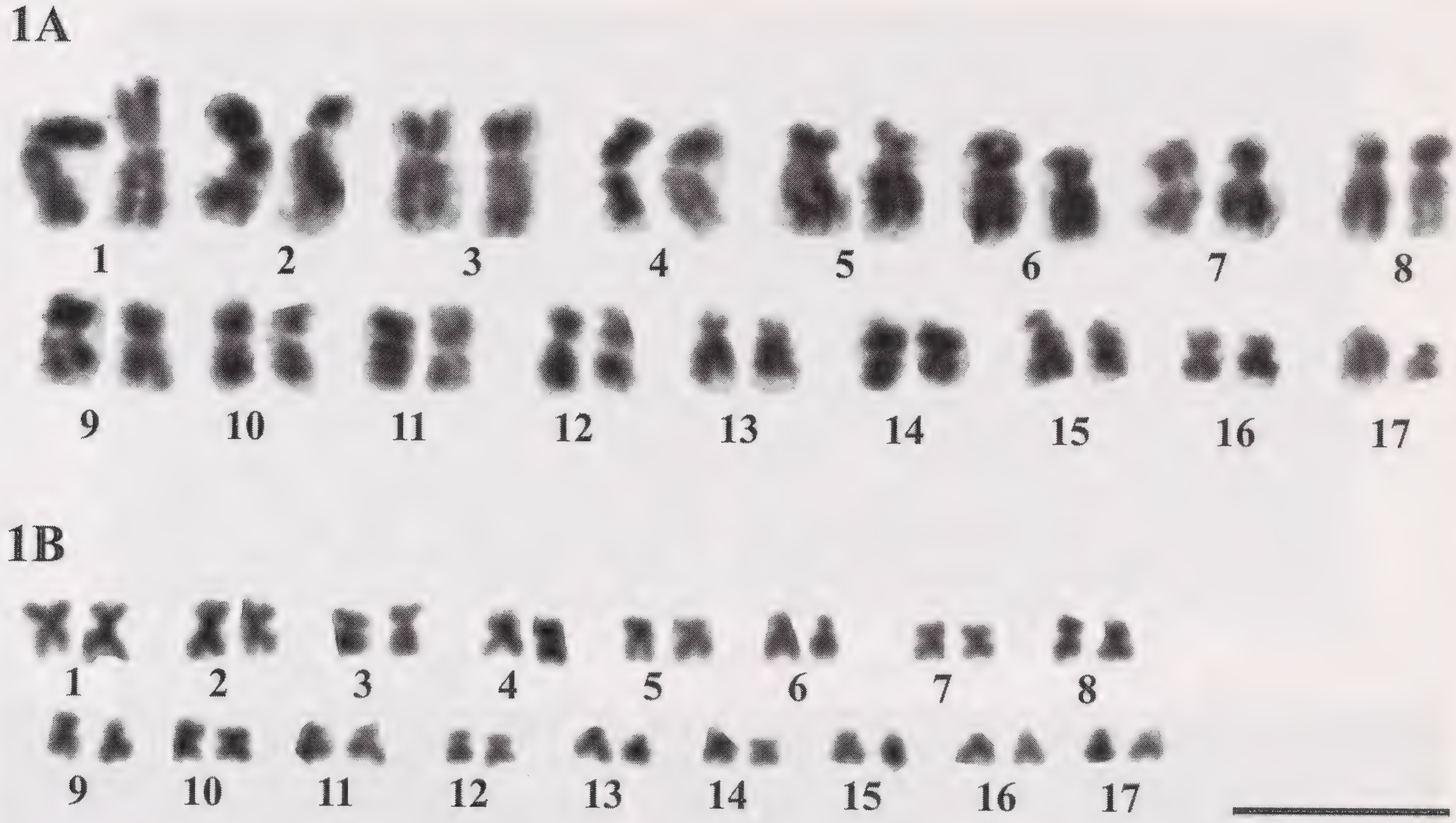


FIG. 1. Giemsa stained karyotypes: *C. unguiformis* (1A – pairs 2, 5, 8, 13, 15, and 17 are subtelocentric; the rest are metacentric/submetacentric,) and *N. stercusmuscarum* (1B – pairs 6, 11, 13, 16, and 17 are subtelocentric; the rest are metacentric/submetacentric). Scale bar = 10 µm.

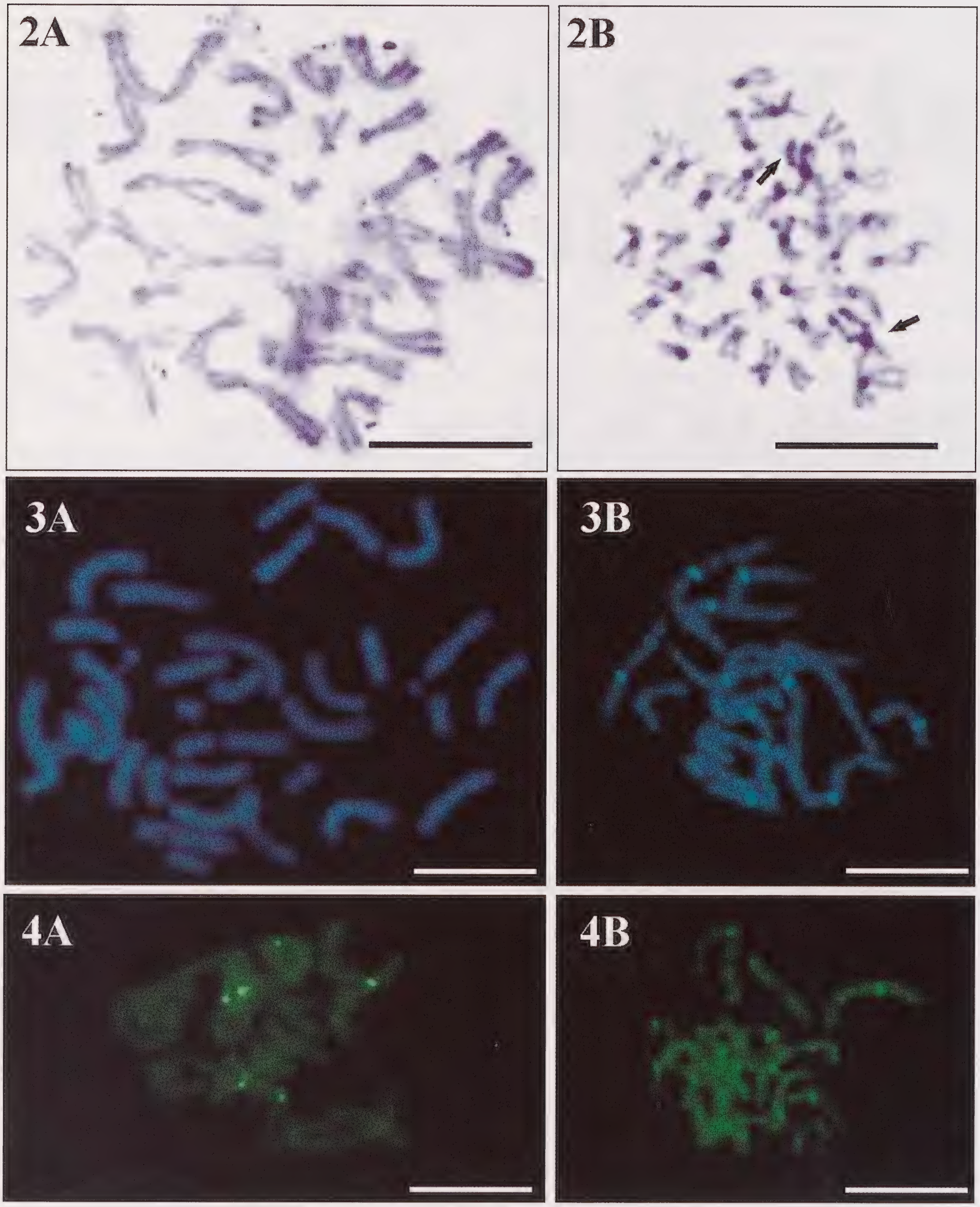
regions were visible on four early diakinetic bivalents. In *N. stercusmuscarum*, brighter spots could be observed in correspondence of heterochromatin after both DAPI (Fig. 3B) and CMA_3 (Fig. 4B) staining. Such a double positive fluorescence of heterochromatin seems unreliable, more probably depending on the high degree of DNA condensation rather than on the real DNA base-pair compartmentalisation.

In *C. unguiformis*, 18S rDNA FISH showed small interstitial hybridization signals near the centromeric region in eight spermatogonial chromosomes (14 metaphases analysed) (Fig. 5A) or in four early diakinetic bivalents (26 plates) (Fig. 6A). In bivalents, location of 18S rDNA is similar to CMA_3 brighter spots indicating that major ribosomal gene sites correspond to GC-rich DNA zones. In *N. stercusmuscarum*, major ribosomal sequences were mapped only on meiotic chromosomes and always appeared terminally located on one bivalent (42 plates) (Fig. 6B).

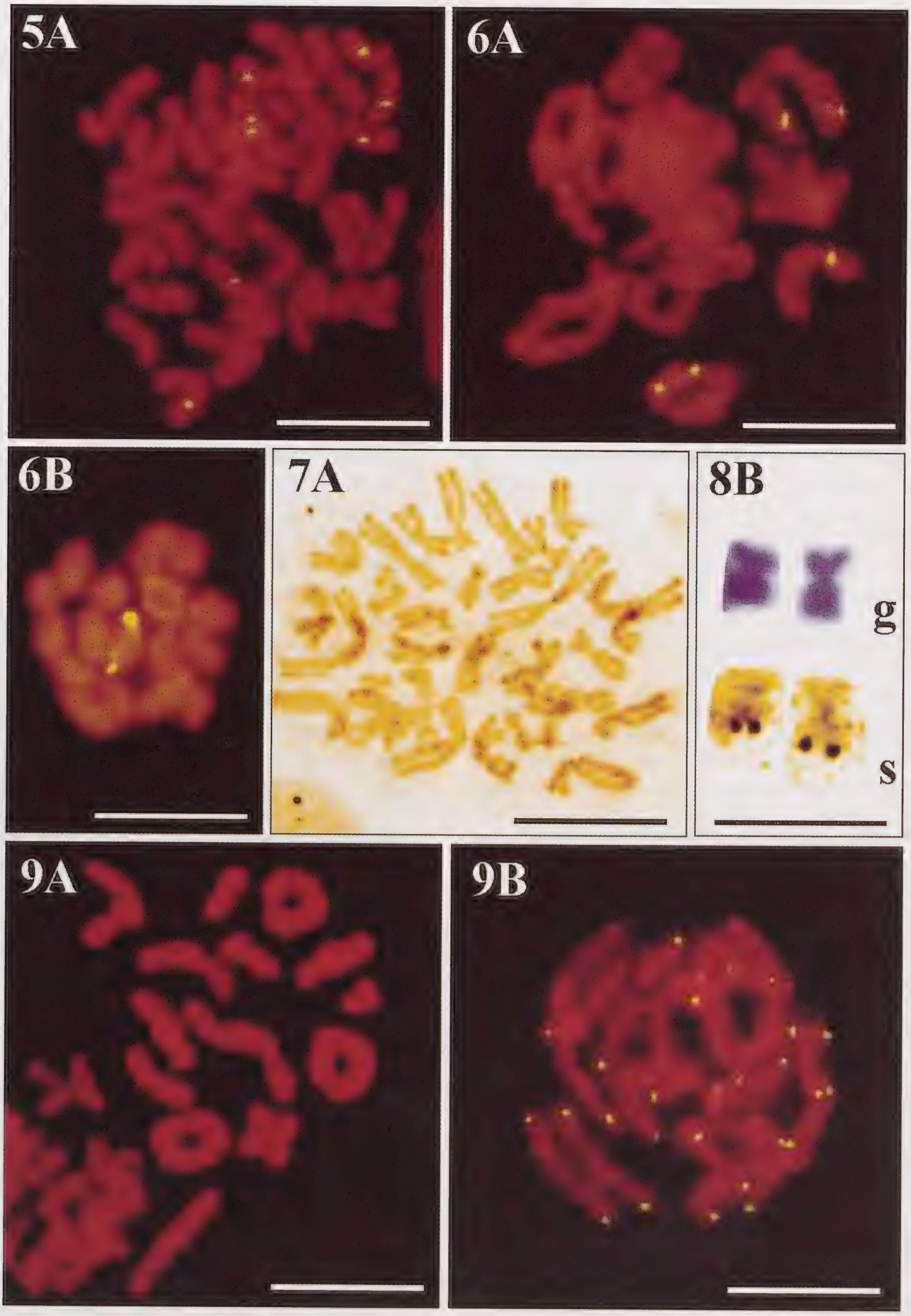
Ag-NOR banding did not provide repetitive argentophilic patterns in *C. unguiformis* (35 metaphases) (Fig. 7A). It is conceivable that the small-size of Ag spots, below the detection threshold of the silver impregnation technique, made difficult disclosure of 18S–28S rDNA clusters.

In 45 spermatogonial metaphases of *N. stercusmuscarum*, dark argentophilic spots were located in the terminal region of two large-sized elements (Fig. 8B), assigned to pair 3 of the karyotype (the same one interested by telomeric heterochromatin blocks).

In *N. stercusmuscarum*, major rDNA sites mapped on the terminal region of a single chromosome pair (two NORs), as already described for other species of Caenogastropoda (Colomba et al., 2002; Vitturi et al., 2002). Differently, in *C. unguiformis*, NORs were located in the interstitial region near the centromere of four chromosome pairs (eight NORs). If we accept the assumption that one pair of terminal NORs per cell is the plesiomorphic condition in both vertebrates (Schmid, 1978) and invertebrates, including molluscs (Thiriot-Quievreux & Insua, 1992; Pascoe et al., 1996), then, we are to conclude that an increase in NOR number has occurred in *C. unguiformis*, whereas the ancestral character (two NORs) has been conserved in *N. stercusmuscarum*. As concerns the mechanism responsible for such an increase, transposition of ribosomal cistrons either by non-homologous exchange or by NOR associated transposons could be the most probable rearrangement (Woznicki et al., 2000) responsible for the multiplication



FIGS. 2–4. C-banded spermatogonial metaphases of *C. unguiformis* (2A) and *N. stercusmuscarum* (2B), arrows indicate the NOR-bearing homologues (pair 3). DAPI stained plates: spermatogonial metaphase in *C. unguiformis* (3A) and spermatocytal pachytene in *N. stercusmuscarum* (3B). CMA_3 stained plates: early diakinetic bivalents in *C. unguiformis* (4A) and pachytene bivalents in *N. stercusmuscarum* (4B). Scale bars = 10 µm.



FIGS. 5–9. *In situ* hybridization with 18S rDNA (from *Paracentrotus lividus*) to spermatogonial metaphase (5A) and early diakinetic bivalents (6A) in *C. unguiformis* and late diakinetic bivalents in *N. stercusmuscarum* (6B). Silver stained spermatogonial metaphase (7A) in *C. unguiformis* and the NOR-bearing chromosomes after Giemsa (g) and silver (s) staining in *N. stercusmuscarum* (8B). $(TTAGGG)_n$ FISH: late diakinetic bivalents in *C. unguiformis* (9A) and pachytene bivalents in *N. stercusmuscarum* (9B). Scale bars = 10 μm.

and dispersion of ribosomal regions. Nevertheless, the mechanism generating interstitial rDNA clusters from the ancestral terminal ones remains unclear. In our opinion, the intrachromosomal position may be hypothesized as the result of subsequent inversions involving NOR-bearing chromosomes.

$(TTAGGG)_n$ FISH

In *C. unguiformis*, the telomeric $(TTAGGG)_n$ probe did not label any chromosomal areas – neither terminal nor interstitial (Fig. 9A). Conversely, typical hybridized areas were clearly detectable at the ends of *N. stercusmuscarum* chromosomes (Fig. 9B). Since slides from both species were treated in the same FISH experiments (five replicates) and/or at the same experimental conditions, we rule out any technical bias underlying this finding.

The "vertebrate" telomere motif $(TTAGGG)_n$ is considered the ancestral telomere repeat motif of Metazoa, conserved in most animal phylogenetic lineages with the up-to-date known exception of two major lineages: Arthropoda (with the TTAGG motif) and Nematoda (with TTAGGC/TTAGCA) (Sakai et al., 2005; Traut et al., 2007, and references therein). Nevertheless, in some species of molluscs, insects, spiders and annelids, telomeres do not hybridize with the expected telomeric probes and, at present, their sequences remain unidentified (Colomba et al., 2000; Vitturi et al., 2000; Frydrychova et al., 2004; Vitkova et al., 2005). $(TTAGGG)_n$ is a widespread (i.e., Guo & Allen, 1997; Plohl et al., 2002; Colomba et al., 2002; Vitturi et al., 2005; Traut et al., 2007; present paper) but not the only motif of molluscan telomeres, since two species, the sacoglossan *Oxynoe olivacea* (Vitturi et al., 2000) and the caenogastropod *Crepidula unguiformis* (present paper) resulted as negative to FISH with this telomeric probe. Such a result might indicate that the telomeric motif $(TTAGGG)_n$ was lost repeatedly and probably replaced with another repeat still unknown.

Genome Size

The mean C values of GS of *C. unguiformis*, *N. stercusmuscarum*, and *H. trunculus* were determined as 6.36 ± 0.11 pg, 2.63 ± 0.11 pg, and 2.31 ± 0.09, respectively. *C. unguiformis* has more than twice the genome size of *N. stercusmuscarum*. Validity of the two data has been confirmed by the comparison with the C-value assessed herein for *H. trunculus* (2.31 pg), very close to 2.26 pg previously determined with Feulgen densitometry on gill cells of this species by Pascoe et al. (2004). The ratio between the GSs of *C. unguiformis* and *N. stercusmuscarum* is roughly corresponding to the ratio between their ranges of chromosome lengths (7.8–2.5 µm *vs.* 3.5–1.2 µm). In comparison with other Neotaenioglossan species (reference in Gregory, 2008), *C. unguiformis* and *N. stercusmuscarum* are endowed by the largest genome sizes. Although slightly larger, the value in *N. stercusmuscarum* resembles 2.40 pg assessed for another moon shell, *Polinices* (*Lunatia*) *heros* Say, 1822, by Hinegardner (1974). Conversely, *C. unguiformis* with 6.36 pg has one of the largest genome sizes up-to-now ascribed to any gastropod molluscs, only lower than four GS values found in the architaenioglossan Diplommatinidae, ranging from 6.70 to 8.04 pg (Ieyama & Ogaito, 2000).

Fluorochrome staining patterns showed a substantial homogeneity in base-pair (AT/GC) composition of nuclear DNA in the two species investigated. In particular, as also revealed by C-banding, *C. unguiformis* has a low amount of heterochromatin and all chromosomes show a dull staining with AT- or GC- fluorochrome, with the exception of NORs, being the only CMA_3 positive areas. Therefore, accumulation of repetitive DNA sequences (heterochromatin) may be excluded in *C. unguiformis* (unless repeated DNA escapes demonstration by standard methods here employed). For this reason, we suggest that euchromatic DNA may be involved in the increase of genome size observed in this species.

CONCLUSION

At a macroscopic scale, inter-specific karyological similarities (i.e., the same diploid number 2n = 34 and the lack of any differentiated sex chromosome systems) can be found in the complements of *C. unguiformis* and *N. stercusmuscarum*. Nevertheless, the application of more powerful methods of investigation, as banding and *in situ* hybridization techniques, along with a comparison between present and compiled data on Caenogastropoda (reviewed in Thiriot-Quievreux, 2003; Gregory, 2008) reveal that *C. unguiformis* is cytogenetically differentiated in: (1) telomeric DNA composition, (2) rDNA clusters number and location, and (3) nuclear DNA content.

Although *C. unguiformis* and *N. stercusmuscarum* are phylogenetically distant they both belong to the Hypsogastropoda, a suborder shown as monophyletic by several molecular analyses (Colgan et al., 2007, and reference therein). Moreover, within the Hypsogastropoda, by comparison of multi-gene sequences Colgan et al. (2007) differentiated the family Calyptraeidae from a set of families called as the "GC group". Present investigation also highlighted that the Calyptraeidae *C. unguiformis* underwent peculiar genomic changes by comparison with known cytogenetical data for the "GC group" families Littorinidae, Naticidae, Vermetidae, and Pterotracheidae (Vitturi et al., 1993, 1995, 1997; Thiriot-Quievreux & Seapy, 1997; Colomba et al., 2002; present paper). Nevertheless, future investigations on other Hypsogastropoda – with special reference to Calyptraeidae – will allow to understand if the cytogenetic diversity of *C. unguiformis* is limited to species- or genus-level or, otherwise, is shared with other species in the same family or suborder.

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ANNUAL REPRODUCTIVE CYCLE AND SIZE AT FIRST SEXUAL MATURITY OF THE SUN AND MOON SCALLOP *AMUSIUM JAPONICUM JAPONICUM* (GMELIN, 1791) (BIVALVIA: PECTINIDAE) IN THE COASTAL WATERS OF JEJUDO, KOREA

Pal-Won Son[1] & Ee-Yung Chung[2]*

ABSTRACT

Gonadosomatic index (GSI), annual reproductive cycle, monthly changes in oocyte size-frequency distributions, size at first sexual maturity and sex ratio of the sun and moon scallop, *Amusium japonicum japonicum,* were investigated. Samples were collected monthly from the subtidal zone of Sogwipo, Jejudo, Korea.

Amusium japonicum japonicum is dioecious. The ovary is composed of a number of oogenic follicles, and the testis consists of a number of acini. Monthly variation in the GSI showed a pattern similar to that of the annual reproductive cycle. Ripe oocytes were about 70–90 μm in diameter. The spawning period was from November to January, and the main spawning occurred between December and January when the seawater temperature was relatively low. The annual reproductive cycle of this species can be classified into five successive stages: early active stage (April to June), late active stage (June to September), ripe stage (October to November), spawning stage (November to January), and spent / resting stage (February to April). The spawning period occurred once per year.

The percentage of first sexual maturity of female and male scallops ranging in length from 85.1 to 90.0 mm was over 50% and 100% for scallops over 90.0 mm length. In the population under study, sexually mature female and male shell lengths (50% of sexual maturity) were 86.96 and 86.59 mm respectively.

The sex ratio of individuals over 85.1 mm length was not significantly different from 1:1 ($\chi^2 = 0.18$, $p > 0.05$). No hermaphrodites were found.

Key words: *Amusium japonicum japonicum*, annual reproductive cycle, size at first sexual maturity, sex ratio.

INTRODUCTION

The sun and moon scallop, *Amusium japonicum japonicum* (Gmelin, 1791), is one of the commercially important edible scallops in East Asian countries, including Korea, China, and Japan (Yoo, 1976; Kwon et al., 1993).

The species is mainly found subtidally along the coast of Jejudo, a major southern island in Korea, inhabiting sandy bottoms to 20–40 m. As a consequence of reckless over-harvesting, the standing stock of this scallop has dramatically declined in recent years (Son et al., 1996) and the species has been denoted as a target organism and fisheries resource that should be managed using a more reasonable fishing regime (Son, 1997). For propagation and management, it is important to understand the population characteristics with regard to the reproductive cycle and size at first sexual maturity.

Several studies have examined aspects of reproduction in *Amusium* spp., including reproductive biology in *A. japonicum balloti* (Dredge, 1981), larval development (Rose et al., 1988), induced spawning and larval rearing (Beldam & del Norte, 1988; Chaitanawisuti & Menasveta, 1992), large–scale hatchery production techniques (Cropp, 1993), and aspects of ecology including growth and recruitment (Williamas & Dredge, 1981), daily growth rings (Joll, 1988), and swimming behaviour (Morton, 1980).

Regarding *Amusium japonicum japonicum*, several studies have focused on reproduction, reproductive ecology and early development (Ha, 1994), aquaculture including age and

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growth (Son et al., 1996), and spat production (Son et al., 1998). Despite the studies referred above, little information on the reproductive cycle, size at first sexual maturity, and the sex ratio of this species is available. The knowledge of the reproductive cycle, spawning period, and temporal variation in the oocyte size-frequency distributions of *A. japonicum japonicum* will provide necessary information for the determination of age and recruitment period and for the estimation of the spawning frequency during the annual spawning period. Additional information on size at first sexual maturity (rate (50%) of sexual maturity) and the sex ratio of this species would be very useful for propagation, aquaculture, and resource management. In particular, information on the size at which individuals reach first sexual maturity could be useful in determining a prohibitory measure for adequate natural resource management. In the present study the annual reproductive cycle, spawning period, size at first sexual maturity, and sex ratio of *A. japonicum japonicum* were studied.

MATERIALS AND METHODS

Sampling Methodology

Specimens of the sun and moon scallop, *A. japonicum japonicum* (Gmelin, 1791), were collected monthly by dredging in the subtidal zone of Sogwipo, Jejudo, Korea, from June 1997 to May 1999 (Fig. 1). A total of 923 scallops ranging from 20.2 to 98.1 mm length were collected during the study. Scallops were transported alive to the laboratory and gonad weight and total meat weight were measured. Unpublished data of seawater temperatures measured daily at 10:00 a.m. at South Jeju Fisheries Hatchery in Sogwipo were used for this study.

Gonadsomatic Index (GSI)

To estimate the spawning period, monthly changes in the mean gonadosomatic index (GSI) were calculated using the following formula: GSI = Gonad weight (g) x 100 / Total meat weight (g) (Chung et al., 2005).

Monthly Changes in Oocyte Size-Frequency Distributions

To investigate monthly oocyte size-frequency distributions, in each month about one thousand eggs that were cut centrally cut in the histological sections were measures, and then plotted and graphed using the frequency curve method of Pearse (1965).

Size at First Sexual Maturity

The onset of first sexual maturity was investigated histologically to determine the shell length of those specimens that developed gametes. Percentages of size at first sexual maturity were calculated as the ratio of mature individuals to the number of females and males investigated respectively.

Size at the Rate (50%) of Sexual Maturity (RM_{50})

For the investigation of age composition by size, von Bertalanffy's equation used by Son et al. (1996) is as follows: $SL_t = SL\infty\ (1\text{-}\ e^{-k\,(t\text{-}to)})$. Where SLt: shell length (mm) at age t; SL∞: theoretical maximum shell length; k: constant expressing rate of approach to SL∞; t: age t; to: theoretical age at which SL = 0 mm; TW: total weight (g) at age t.

To calculate the size at the rate (50%) of sexual maturity after fitting the rate of sexual maturity to an exponential equation, the size equivalent to the size at 50% of sexual maturity was estimated to be the sexually mature length of the population (Chung & Ryou, 2000). The exponential equation of the rate of sexual maturity is as follows: $RM = 100 / 1 + \exp^{(a-bx)}$, where, RM: rate of sexual maturity; a, b: constants; x: shell length.

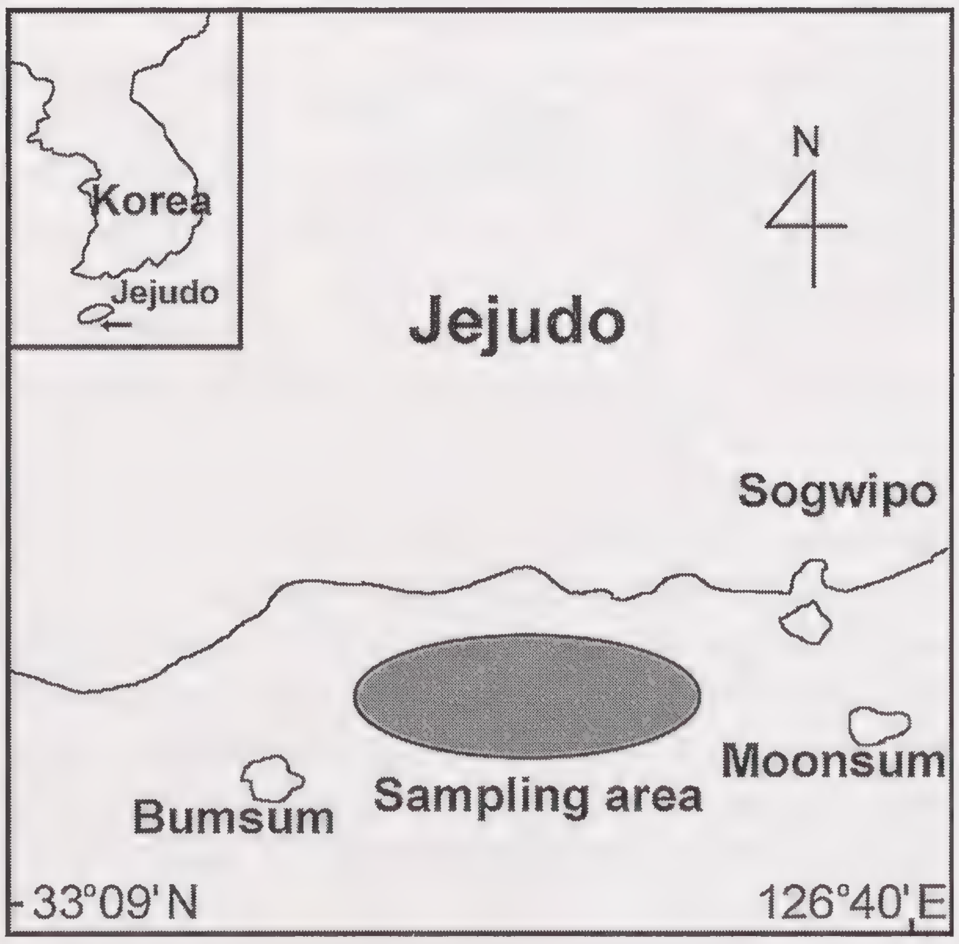

FIG. 1. Map showing the sampling area.

Sex Ratio

The sex ratio of *Amusium japonicum japonicum* was estimated from monthly samples obtained from June 1997 to May 1998; the sex of 461 sexually mature individuals > 85 mm shell length was determined by microscopic examination of histological sections. A Chi square, goodness-of-fit test was used to test the hypothesis of equal representation of females and males.

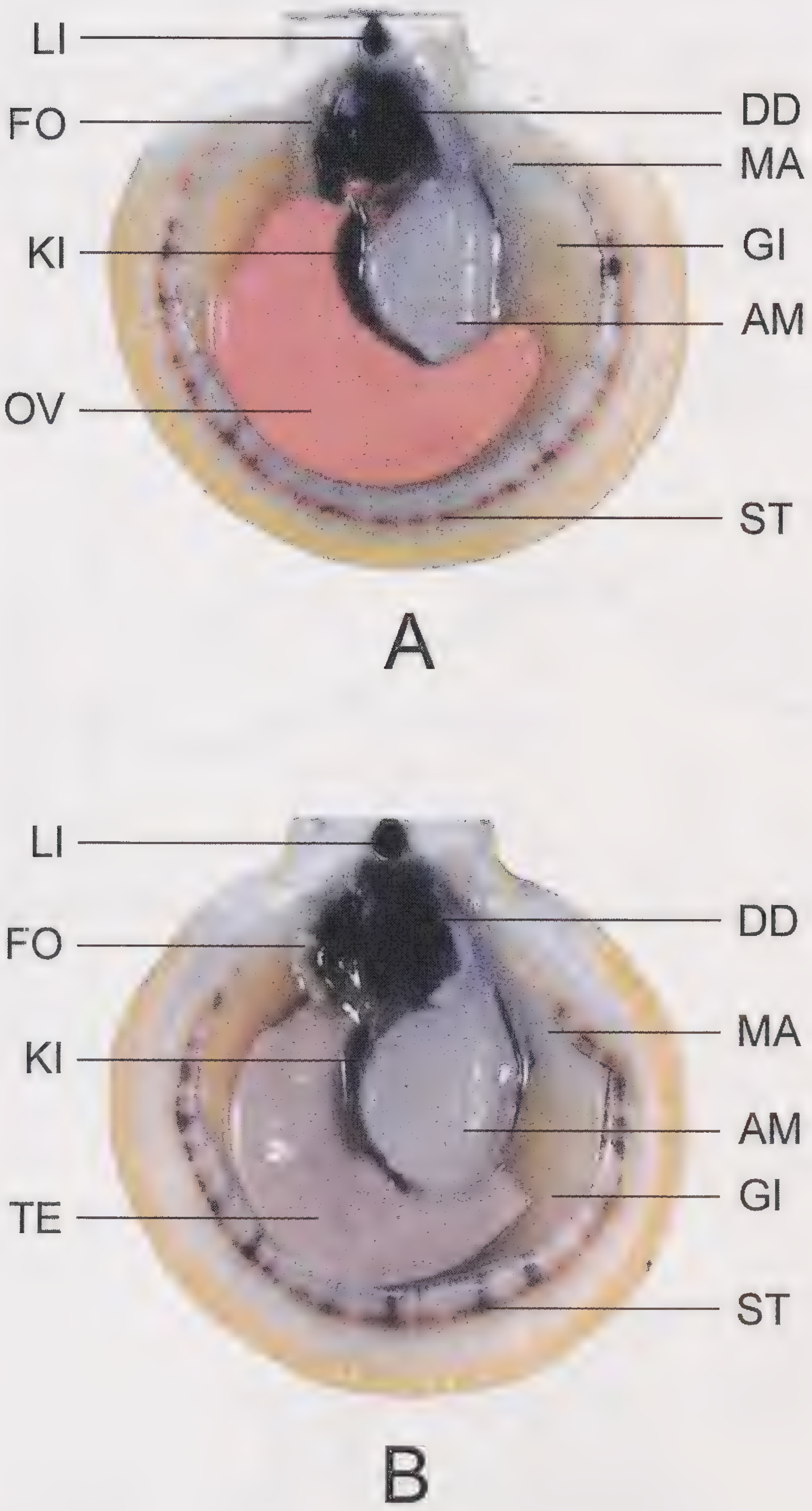

FIG. 2. Organs of mantle cavity from right side of *Amusium japonicum japonicum*, with right shell, mantle and gill removed. A: female; B: male. Abbreviations: AM, adductor muscle; DD, digestive diverticulum; FO, foot; GI, gill; KI, kidney; LI, ligament ; MA, mantle; OV, ovary; ST, sensory tentacles; TE, testis.

RESULTS

External Morphology of the Gonads

Amusium japonicum japonicum is dioecious. Gonads are conical or crescent-shaped and are well separated from the digestive diverticula lying at side of the adductor muscle. As maturity progresses, gonads encircle the adductor muscle near digestive diverticula (Fig. 2). When the gonads are mature, the external colour of the ovary is pink and that of testis is milky white or light yellow. Therefore, in sexually mature specimens the sexes can be easily determined by direct observation. After spawning, undischarged germ cells degenerate, and then it becomes difficult to distinguish the sexes by external color.

Monthly Changes in the Gonadosomatic Index (GSI) and Seawater Temperatures

Monthly GSI changes in females and males are shown in Figure 3. In 1997–1998, the values of GSI corresponding to both females and males began a gradual increase in July, reaching the maximum (females mean GSI 22.2 ± 2.5; males mean GSI 15.0 ± 1.7) in November, when seawater temperature gradually decreased. Then, the GSI rapidly decreased from December to January when relatively low water temperatures were maintained and spawning occurred continuously. Thereafter, the mean GSI value temporarily reached a minimum in May 1998 (3.4 ± 0.8) in female and June 1998 (2.5 ± 0.8) in male scallops. Monthly changes in the GSI in females and males in 1998 showed a similar pattern.

Monthly Changes in the Oocyte Size-Frequency Distributions

To determine the spawning period and the number of spawning frequencies, relative oocyte size-frequency was measured using histological sections. Monthly changes in oocyte size-frequency distributions from June 1997 to May 1998 are shown in Figure 4. Percentages of oocytes measuring more than 50 μm in diameter increased between June and July 1997. Between August and September, ripe ova measuring 70 μm in diameter increased in number.

In October 1997, the number of ripe ova rapidly began to increase and the relative frequency of ripe ova ranging from 70 to 90 µm in diameter was over 60%. In November 1997, when spawning began, the number of ripe ova measuring 70–90 µm began to decrease, however the size class of smaller egg diameter (< 20–30 µm) grew larger (> 40–60 µm). The peak spawning of ripe ova, measuring 70–90 µm in diameter, occurred continuously between December 1997 and January 1998. Thereafter, the number of ripe ova with large diameters decreased considerably in number (because of their discharge). From February to May, the small number of large undischarged oocytes, < 50–70 µm in diameter, began to degenerate and many oogonia and oocytes measuring 10–20 µm in diameter remained in the oogenic follicles.

Annual Reproductive Cycle with the Gonadal Phases

Based on morphological features and sizes of the germ cells and accompanying cells, the annual reproductive cycle of gonadal phases can be classified into five successive stages (Figs. 5, 6).

Early Active Stage: In females, oogonia and previtellogenic oocytes proliferate along the follicular wall (germinal epithelium) in the ovary. The oogonia and previtellogenic oocytes are 9–11 µm and 15–25 µm in diameter, respectively. Also some auxiliary cells, which are attached to the previtellogenic oocyte, appear near the follicular wall of the oogenic follicle (Fig. 5A).

In males, spermatogenesis occurs in acini. The spermatogonia and spermatocytes are 8–9 µm and 6–7 µm in diameter, respectively, and they appear along the acinus wall (Fig. 6A).

Female and male individuals in the early active stage appear from April to June when seawater temperatures are gradually increasing.

Late Active Stage: In females, a number of the early vitellogenic oocytes, ranging from 30

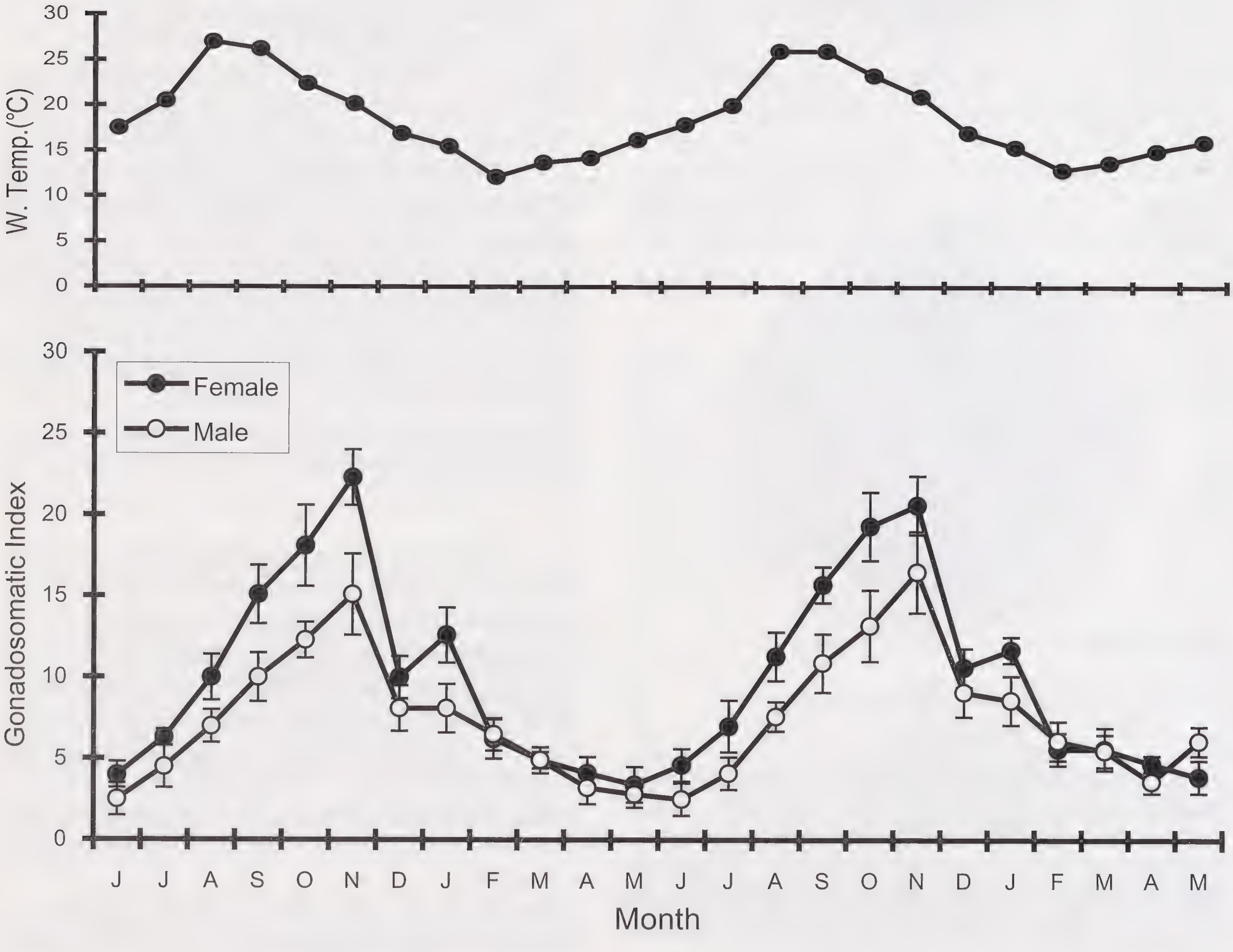

FIG. 3. Monthly variations of male and female gonadosomatic index (GSI) and mean water temperature, for two years from June 1997 to May 1999. The vertical bars indicate standard deviation (SD).

to 45 μm in diameter, appear in the oogenic follicles. Each oocyte has an egg-stalk attached to the follicular wall and the nucleus is enlarged, having a small nucleolus. At this time, some auxiliary cells, which were attached to the stalk region of an early vitellogenic oocyte, appear near the follicular wall. When late vitellogenic oocytes grow to 50–65 μm in diameter, the cytoplasm of the oocytes are filled with a number of yolk granules (Fig. 5B).

In males, a few spermatogonia and spermatocytes are present near the acinus wall. However, a small number of spermatids, measuring 3–4 μm in diameter, are also found in the lumen of the acinus. At this time a few spermatids begin to transform into differentiated spermatozoa in the centre of the lumen. As spermatogenesis progresses, the number of spermatocytes, spermatids and the few spermatozoa occupy approximately one-third to one-half of the lumina of the acini (Fig. 6B). Female and male individuals in the late active stage are found from June to September when seawater temperatures are relatively high.

Ripe Stage: In females, the ripe ova are 70–90 μm in diameter, and are round or oval in shape. They are located in the centre of the lumen. At this time, ripe ova have a large germinal vesicle; are surrounded by a thick egg envelope. The cytoplasm of the ripe ovum is filled with a number of yolk granules (Fig. 5C).

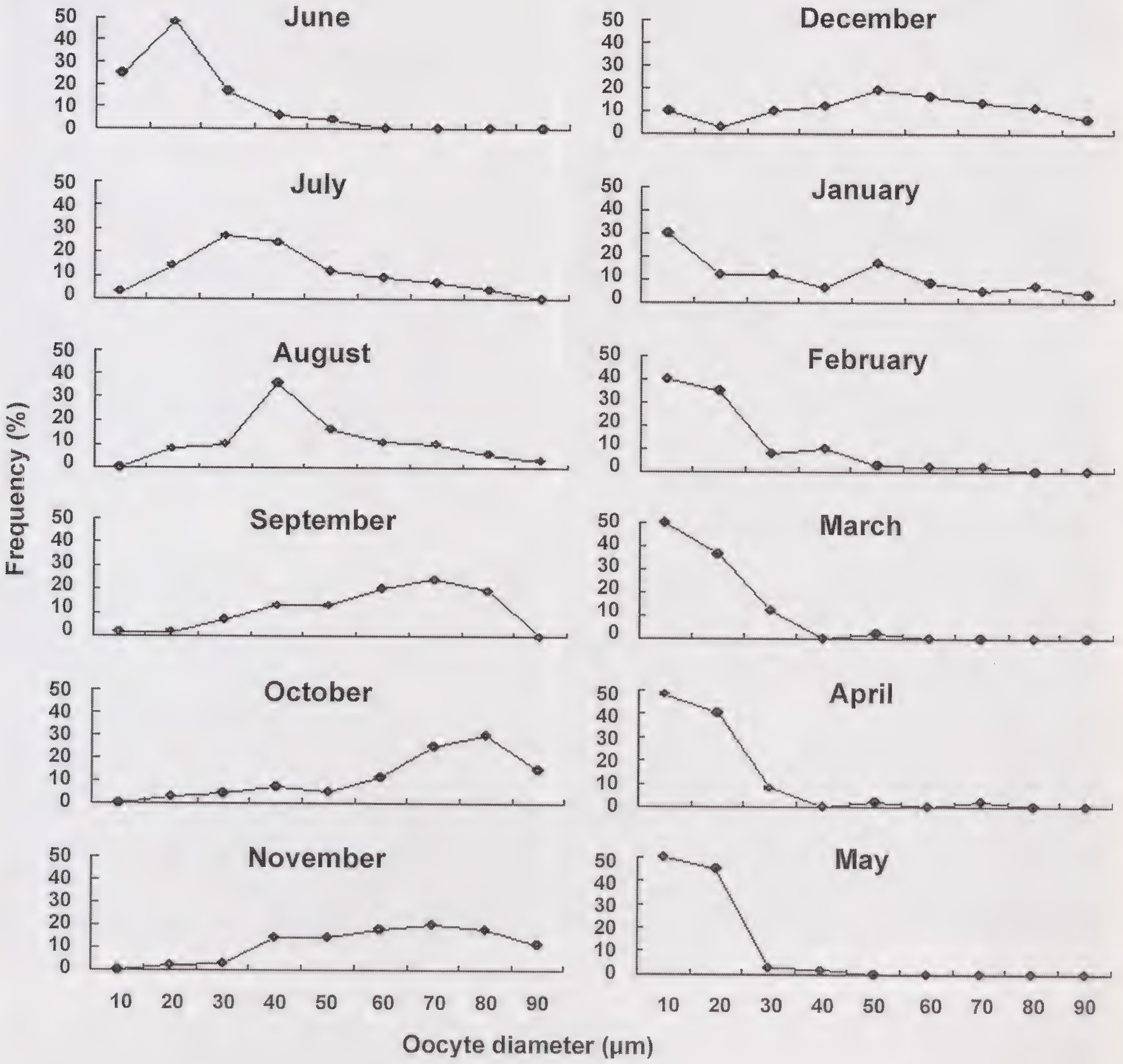

FIG. 4. Monthly changes in oocyte size-frequency distributions of *Amusium japonicum japonicum* from June 1997 to May 1998.

In males, a small number of spermatids begin to transform into differentiated spermatozoa in the centre of the lumen and numerous spermatozoa appear in the center of the lumen of the acinus (Fig. 6C). Ripe gonads are found between October and November when seawater temperatures are gradually decreasing.

Spawning Stage: In females, 50–60% of the ripe ova in the oogenic follicles have been discharged. The lumen is largely empty. Spawned ovaries are characterized by the presence of a few ripe, undischarged and previtellogenic oocytes in the lumen (Fig. 5D). In males, the lumina of the acini are empty because over 50%

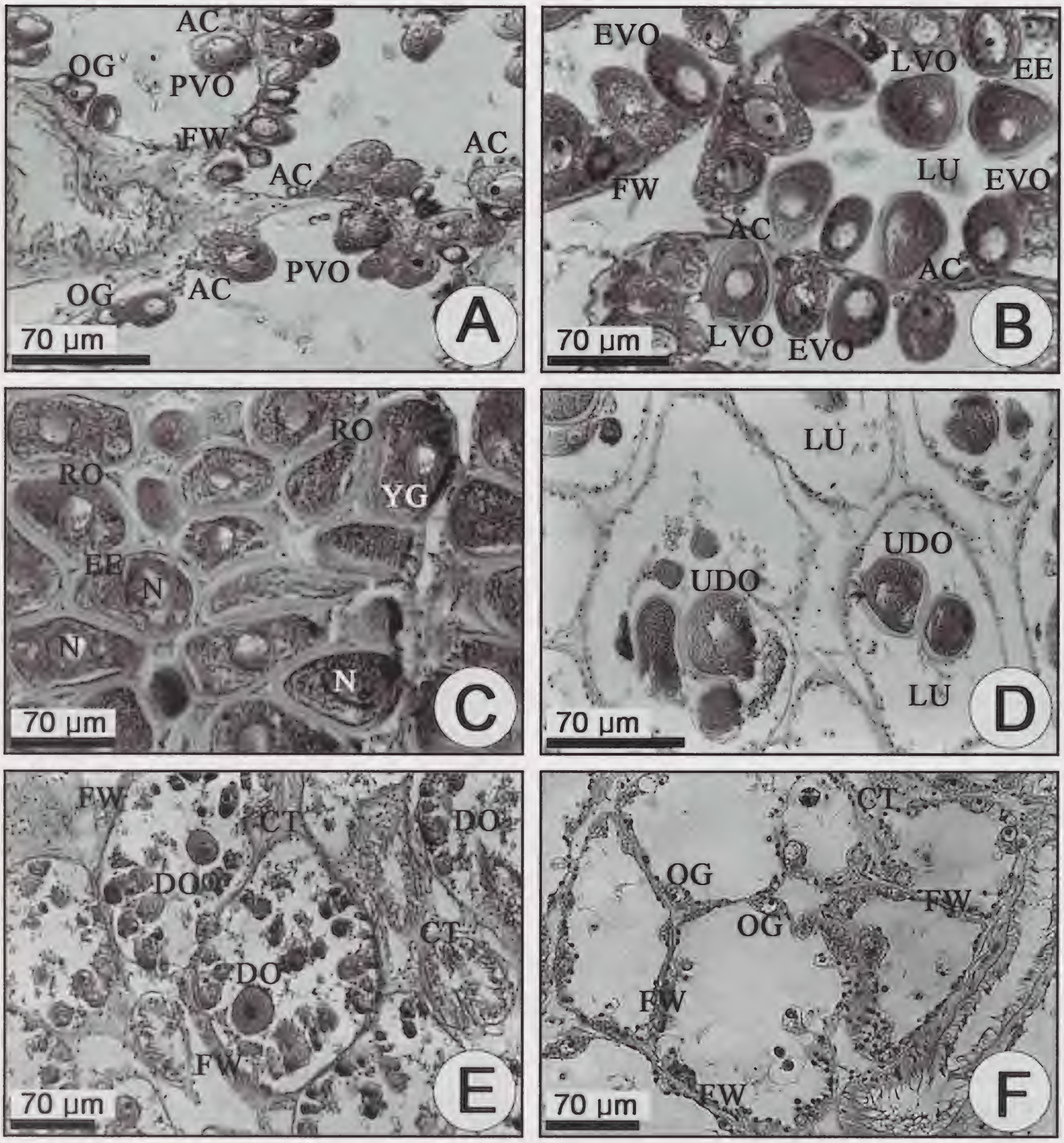

FIG. 5. Photomicrographs showing the gonadal phases in female *Amusium japonicum japonicum*. A: early active stage; B: late active stage; C: ripe stage; D: spawning stage; E, F: spent / resting stage. Abbreviations: AC, auxiliary cell; CT, connective tissue; DO, degenerating oocyte; EE, egg envelope; EVO, early vitellogenic oocyte; FW, follicular wall; LU, lumen; LVO, late vitellogenic oocyte; N, nucleus; OG, oogonium; PVO, previtellogenic oocyte; RO, ripe ovum; UDO, undischarged oocyte; YG, yolk granule.

of the spermatozoa have been discharged, but undischarged spermatozoa as well as spermatids remain in the lumen of the acinus (Fig. 6D). Individuals in the spawning stage appear from November through January, with the main spawning event occurring between December and January when seawater temperatures are relatively low.

Spent / Resting Stage: In females, after spawning, the undischarged oocytes in the lumen undergo cytolysis and the products of gamete atresia are resorbed (Fig. 5E). Thereafter, rearrangement of some new oogonia and connective tissue appeared in the oogenic follicles (Fig. 5F). In males, the few remaining spermatozoa and spermatids degenerate and

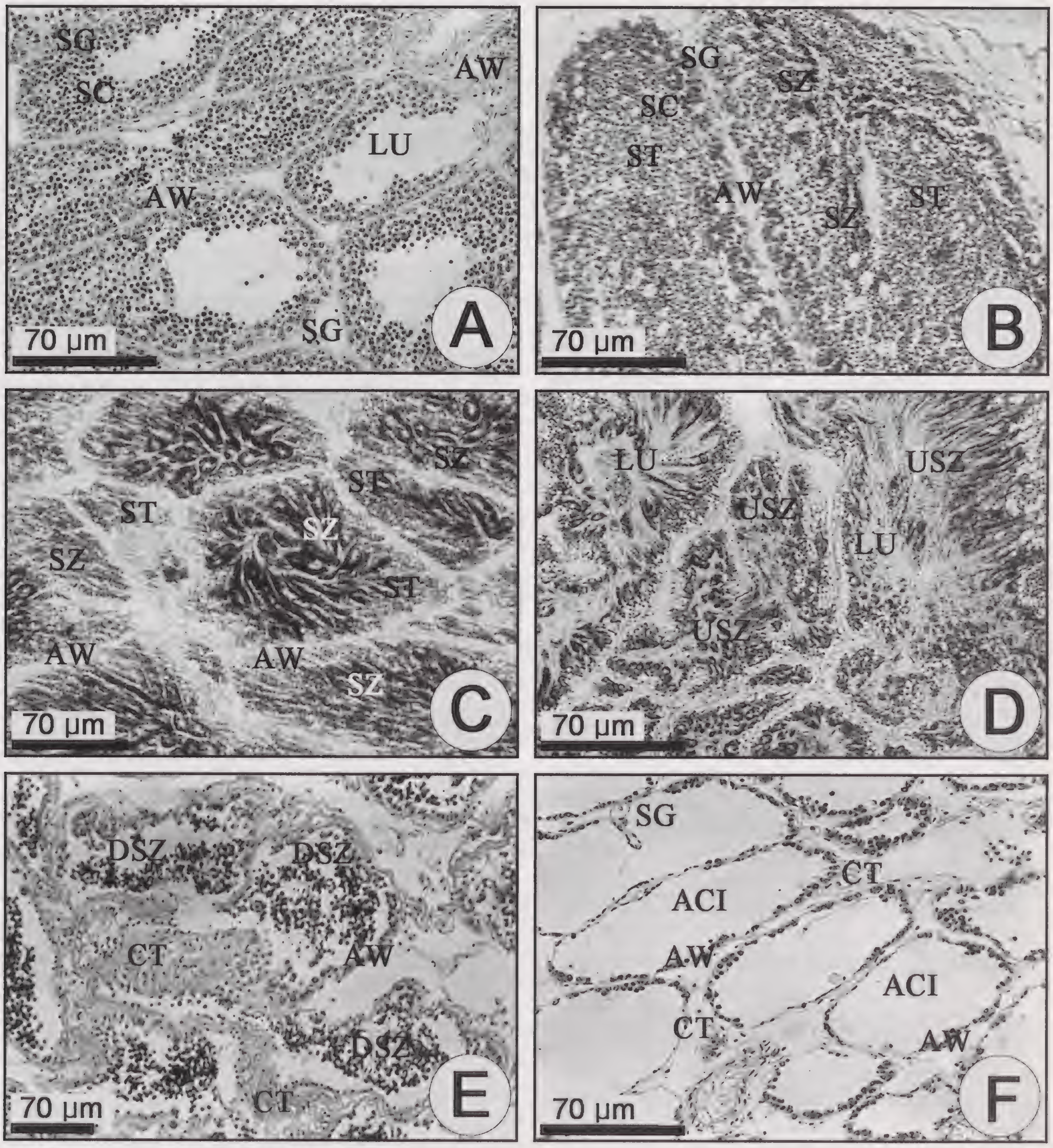

FIG. 6. Photomicrographs showing the gonadal phases in male *Amusium japonicum japonicum*. A: early active stage; B: late active stage; C: ripe stage; D: spawning stage; E, F: spent / resting stage. Abbreviations: ACI, acinus; AW, acinus wall; CT, connective tissue; DSZ, degenerating spermatozoon; LU, lumen; SC, spermatocyte; SG, spermatogonium; ST, spermatid; SZ, spermatozoon; USZ, undischarged spermatozoon.

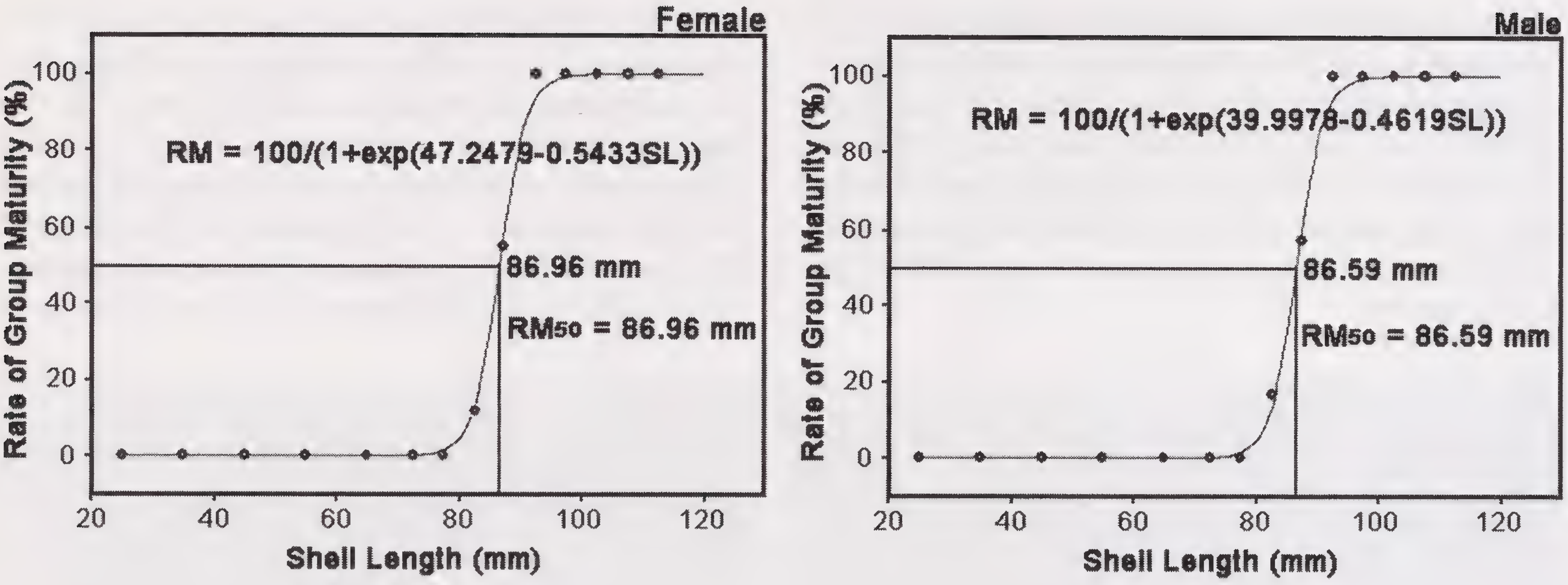

FIG. 7. Relationship between the rate of sexual maturity (%) and shell length (mm) in male and female *Amusium japonicum japonicum*.

the products of gamete atresia are resorbed (Fig. 6E). Thereafter, the rearrangement of a few newly formed spermatogonia and connective tissue occur in the acini (Fig. 6F). Female and male individuals in this stage are found from February to April when seawater temperatures are relatively low.

Size at First Sexual Maturity

A total of 171 (90 females and 81 males) individuals of *Amusium japonicum japonicum* were investigated histologically to determine the shell lengths of scallops that reach maturation and participate in reproduction from October (before spawning) to late February (after spawning). As shown in Table 1, the percentage of first sexual maturity of female and male scallops ranging in length from 85.1 to 90.0 mm were over 56%, and this was 100% for scallops over 90.1 mm length. According to the growth curves for the mean shell length fitted to von Bertalanffy's equation, individuals ranging from 85.1 to 90.0 mm in shell length were considered to be about two years old.

TABLE 1. The shell length at first sexual maturity in *Amusium japonicum japonicum.*

Shell length	Female		Male	
(mm)	Number	Mature (%)	Number	Mature (%)
20.2–30.0	4	0	4	0
30.1–40.0	7	0	5	0
40.1–50.0	7	0	4	0
50.1–60.0	6	0	6	0
60.1–70.0	7	0	7	0
70.1–75.0	8	0	6	0
75.1–80.0	8	0	8	0
80.1–85.0	9	11.1	6	16.7
85.1–90.0	9	55.5	7	57.1
90.1–95.0	7	100	6	100
95.1–100.0	7	100	4	100
100.1–105.0	4	100	5	100
105.1–110.0	4	100	7	100
110.1–114.8	3	100	6	100
Total	90		81	

TABLE 2. Monthly variations in sex ratios of the adult clam *Amusium japonicum japonicum* (> 85.1 mm in shell length).

Month	Female (ind.)	Male (ind.)	Total (ind.)	Sex ratio (F/(F+M))	Chi squared
Jun. 1997	17	22	39	0.44	0.64
Jul. 1997	16	20	36	0.44	0.44
Aug. 1997	20	18	38	0.53	0.11
Sep. 1997	22	20	42	0.52	0.10
Oct. 1997	21	19	40	0.53	0.10
Nov. 1997	22	18	40	0.55	0.40
Dec. 1997	20	17	37	0.54	0.24
Jan. 1998	24	18	42	0.57	0.86
Feb. 1998	20	17	37	0.54	0.24
Mar. 1998	21	16	37	0.57	0.68
Apr. 1998	15	21	36	0.42	1.00
May 1998	17	20	37	0.46	0.24
Total	235	226	461	0.51	0.18

Size at the Rate (50%) of Sexual Maturity (RM50)

Shell lengths of sexually mature scallops (sizes at the rate (50%) of sexual maturity, RM_{50}) that fitted to an exponential equation were 87.0 mm in females and 86.6 mm in males (Fig. 7).

Sex Ratio

Of 461 scallops examined, 235 were females and 226 were males (Table 2). There was no statistically significant difference in the numbers of females and males ($\chi^2 = 0.18$, $p > 0.05$). and similarly, monthly comparisons do not show statistical differences. In eight out of twelve months, there were slightly more females than males and opposite was tru for the remaining four months. The sex ratios of individuals over 85.0 mm in shell length were not significantly different from 1:1. No. hermaphrodites were found in histological sections.

DISCUSSION

Several authors (Pipe, 1987; Dorange & Le Pennec, 1989; Dorange et al., 1989; Gaulejac et al., 1995; Eckelbarger & Davis, 1996; Eckelbarger & Young, 1999; Chung et al., 2005) have reported that the auxiliary cells (or follicle cells), which attach to the previtellogenic and the early vitellognic oocytes in the follicle, appeared near the egg stalk region of the oocyte in the early active stage. In the present study, however, the auxiliary cells (follicle cells), which were attached to the previtellogenic and early vitellogenic oocytes, gradually detached from the late vitellogenic oocytes before the completion of the vitelline envelope during oogenesis (Pipe, 1987; Dorange et al., 1989; Gaulejac et al., 1995; Eckelbarger & Davis, 1996; Eckelbarger & Young, 1999; Chung et al., 2005).

Pipe (1987) reported that in *Mytilus edulis* once the follicle cells withdraw, the microvilli appear along the oolemma of the oocyte. In the present study, we observed a similar phenomenon. Therefore, it is assumed that the auxiliary cells, which are attached to the egg envelope of the oocyte, play some role in the formation of the vitelline envelope.

In most bivalve ovaries examined, including those of *Crassostrea virginica* (Eckelbarger & Davis, 1996), several follicle cells were associated with each oocyte during early stages of oogenesis. However, only a single “auxiliary cell” was associated with each oocyte of *Pecten maximus* (Dorange & Le Pennec, 1989; Dorange et al., 1989). In contrast, *Patinopecten yessoensis* (Chung et al., 2005) “auxiliary cells” completely encompassed oocytes (Chung et al., 2005). In the present study, a few auxiliary cells of *A. japonicum japonicum* were associated with each oocyte during early stages of oogenesis, therefore, our results showed a similar pattern to those of *P. maximus* (Dorange & Le Pennec, 1989; Dorange et al., 1989).

By using electron microscopic observation, Pipe (1987) described that the follicle cells, which are attached to the oocyte in *M. edulis*, appeared to play an integral role in oocyte development according to electron microscopic data. However, Galtsoff (1964) doubted that follicle cells played any role in oocyte nutrition in *C. virginica* due to their small size and number. Therefore, there is a need to study the functions of auxiliary cells and the relationship between the oocyte and the auxiliary cells using electron microscopy.

In marine bivalves, cyclical development and resorption of gametes can be regarded as an adaptation to environmental temperature and food availability (Morvan & Ansell, 1988). If the energy required for the production of gametes is too large, nutritive reserves might not be enough to allow all gametes to reach the critical size for spawning. In this case, the products of gamete atresia could be resorbed and the energy reallocated to still-developing oocytes or used for other metabolic purposes (Dorange & Le Pennec, 1989; Mortavkine & Varaksine, 1989).

Our histological observations showed that spawning of *Amusium japonicum* in Jejudo, Korea, occurred from November to January and the spawning peak was observed between December and January. in the present study showed that spawning of *A. japonicum japonicum* in Jejudo, Korea, occurred from November to January, and the spawning peak was observed between December and January. Judging from monthly changes in the oocyte size-frequency distributions (Fig. 4) and artificial spawning experiments in the laboratory (Son et al., 1998), the spawning period is once a year.

The spawning period of *Amusium balloti* (Bernardi, 1861) in Shark Bay, Australia, is between December and January (Heald & Caputi, 1981) and that of *A. pleuronectes* (Linnaeus, 1758) in Thailand is from November to February (Chaitanawisuti & Mennasveta, 1992). Therefore, we suggest spawning of *Amusium* spp. probably generally occurs during the period from November through February. Breeding in bivalves can occur seasonally or year-round, and as in other marine molluscs (e.g., Webber & Giese, 1969), the breeding season is affected by local environmental conditions as well as genetic differences among populations (Chung et al., 1991, 2002; Chung & Ryou, 2000). Regarding breeding habits of marine mollusks, Boolootian et al. (1962) placed mollusks into three large categories: (1) year-round breeders, (2) winter breeders, and (3) summer breeders. We found that *A. japonicum japonicum* belongs to the winter breeder class.

Son et al. (1996) found that *A. japonicum japonicum* reached a maximum shell length of 90.7 mm after two years. In the present study, percentages of first sexual maturity of female and male scallops ranging from 85.5 to 90.0 mm were 55.5% and 57.1%, respectively, and this is 100% for scallops over 90.1 mm shell length.

According to Son et al. (1996), the individuals ranging from 85.0 to 89.9 mm in shell length were considered to be about two years old. We assume that both sexes of this population begin reproduction about two years of age.

In terms of natural resource management, the present study suggests that harvesting scallops less than 85.0 mm in shell length (< 2 years old) can potentially lead to a drastic reduction in recruitment. A prohibitory measure should be taken for adequate natural resources management.

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REDESCRIPTION OF *UNIO GIBBUS* SPENGLER, 1793, A WEST PALAEARCTIC FRESHWATER MUSSEL WITH HOOKLESS GLOCHIDIA

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ABSTRACT

As part on an ongoing effort to unravel the taxonomy and phylogeny of Palaearctic freshwater mussels, this paper describes an overlooked *Unio* species that inhabits the Atlantic rivers of South Iberia and Morocco, based on morphological, reproductive, and molecular characters. Live specimens of *Unio gibbus* were recently found in the Barbate River in southwest Spain and in the Beth River in the Sebou basin, northwest Morocco.

Phylogenetic relationships inferred from partial COI and 16S rRNA gene sequences point to the recognition of *U. gibbus* as an evolutionary unit. Genetic divergences with respect to other *Unio* species ranged from 10.24% and 6.18% (compared to *U. tumidus*) to 12.91% and 8.89% (compared to *U. tumidiformis*) for COI and 16S rRNA respectively.

In the Spanish specimens, the entire internal cavity of the external demibranchs (homogeneity) acts as a marsupium (ectobranchy), yet in the Moroccan population, the four gills are occasionally filled with glochidia (occasional tetrageny). Glochidia are rounded triangular, intermediate between the shapes of those in other species of *Unio* and *Potomida*. The border of the larval shell bears numerous small lumps, but these fail to form the ventral styliform hook typical of the genus *Unio*. These two characters, tetrageny and hookless glochidia, both of major significance in phylogenetic studies of the group, are reported here for the first time in the genus *Unio*.

The Spanish populations of *U. gibbus* should be a main priority for European invertebrate conservation measures.

Key words: *Unio*, hookless glochidia, Iberian Peninsula, Morocco, mitochondrial DNA.

INTRODUCTION

Most specialists would agree that the seminal work of Haas (1969) on unionoid taxonomy forms the basis for any modern study on the taxonomy and phylogeny of this mollusc group. The genus *Unio* was considered by Haas (1969) as a series of 12 "fundamental *Unio* species" comprised of different "races" or incipient species. Although this author's work was mostly based on conchology, Haas laid down the basic framework for current freshwater mussel systematics. In a recent review of the systematics and global diversity of the freshwater mussel species, Graf & Cummings (2007) only considered seven species under the genus *Unio*. Thus, the taxonomy of this polymorphic group now needs to be reconstructed as new molecular data and further biological characters, such as shell morphology, anatomy, glochidium, come to light. Recent studies on European unionoid taxonomy necessitate the formulation of new hypotheses regarding the number of evolutionary independent taxa in this imperilled mollusc group. With Haas' work as the base, recent conchological, anatomical, reproductive and molecular data have revealed several new features of the taxonomy of West Palaearctic naiads: (1) the two Iberian Mediterranean *Unio* discussed by Haas (1969) – *U. elongatulus penchinatianus* Bourguignat, 1865, and *U. elongatulus valentinus* Rossmässler, 1854 – are actually synonyms of *U. mancus* Lamarck, 1819 (Araujo et al., 2005); (2) the two Iberian *Potomida* discussed by Haas (1969) – *P. littoralis littoralis* Cuvier, 1798, and *P. littoralis umbonata* Rossmaessler, 1844 – are synonyms of *P. littoralis* Cuvier, 1798 (Reis et al., in review); (3) *Unio tumidiformis* Castro, 1885, an overlooked Iberian endemic, is being redescribed (Reis & Araujo, in review); and (4) *Margaritifera marocana* Pallary, 1918, has also been redescribed as a valid species that differs from *M. auricularia* (Spengler, 1793) (Araujo et al., in press).

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As part of an ongoing study of the phylogeny of the Palaearctic unionoids, the present paper describes another overlooked *Unio* species based on the character set mentioned above. The species was previously considered a synonymy of the southwest Atlantic Iberian *U. pictorum delphinus* Spengler, one of the 13 taxa into which Haas (1969) separated the west Palaearctic species of *Unio pictorum* (Linnaeus, 1758).

MATERIALS AND METHODS

To determine the historical distribution of the species, we reviewed Spengler (1793), Bourguignat (1864, 1865), Drouet (1893), Simpson (1900), Haas (1913, 1917, 1969), Pallary (1923, 1927, 1936), Azpeitia (1933), Daget (1998), Soriano et al. (2001), and Knudsen et al. (2003). Recent specimens were obtained by field sampling the Iberian Peninsula and Morocco in 2006 and 2007; additional material was kindly provided by collaborators. Specimens were collected by wading the rivers, sometimes using a water-scoop, or by snorkelling. Some specimens (six from Spain and eight from Morocco) were transported alive in the cold (without water) to the laboratory where they were dissected and photographed.

Shell characters were examined in adult and juvenile specimens from the two rivers where the species were found. Detailed descriptions were made of shell shape and outline, colour, umbonal sculpture, and hinge characteristics. All relevant features were photographed.

Anatomical studies were conducted on live and preserved specimens from the two rivers, paying close attention to characters identified by other authors as taxonomically relevant in unionoids (Ortmann, 1911; Haas, 1924; Nagel, 1999; Graf & Cummings, 2006).

To characterize the larval stage, glochidia were collected from live mussels by their separation from gravid gills, cleaned with KOH and gold-coated for scanning electron microscopy.

For molecular analyses, two foot samples from 15 specimens (six from Spain and nine from Morocco) were obtained and preserved both frozen at -75°C and in absolute ethanol. Total DNA was extracted using the ChargeSwitch gDNA Micro Tissue (Invitrogen) extraction kit or classic phenol/chloroform procedures (Sambrook et al., 1989). Partial COI and 16S rRNA gene sequences were amplified by polymerase chain reaction (PCR) using the same primers as in Machordom et al. (2003). The PCR mix contained 3µl DNA, 5µl of the corresponding buffer (with 10x 2mM $MgCl_2$), 1µl of dNTPs mix (10mM), 0.8µl of both primers (10µM), 0.3µl *Taq* DNA polymerase (5U/µl) (Biotools) and ddH_2O in a total volume of 50µl. The amplification conditions for both genes were as follows: 94°C (4 min), 40 cycles of 94°C (45 s), 45°C (1 min), 72°C (1 min), and a final extension at 72°C (10 min).

The products were visualised with blue light in 0.8% agarose gels stained with SYBR Safe (Invitrogen). A co-migrating 100 pb or 1Kb ladder molecular-weight marker served to confirm correct amplification. The amplified fragments (around 700 bp) were purified by ethanol precipitation prior to sequencing both strands using BigDye Terminator (Applied Biosystems, ABI). Products were electrophoresed on an ABI 3730 genetic analyzer (Applied Biosystems). The forward and reverse DNA sequences obtained for each specimen were aligned and checked using the Sequencher program (Gene Code Corporation) after removing primer regions. When necessary, Clustal X (Thompson et al., 1997) was used to align the obtained sequences of 16S, but the final alignment was adjusted by eye.

For the phylogenetic analyses, we selected GenBank *Unio* sequences representing the type localities of various species (or at least populations close to them), such as *Unio pictorum* (Linnaeus, 1758); *U. tumidus* Philipsson, 1788; *U. crassus* Philipsson, 1788; *U. douglasiae* Gray, in Griffith & Pidgeon, 1833; *U. caffer* Krauss, 1848; and *U. mancus* Lamarck, 1819, and those from the Iberian Peninsula studied by Reis et al. (in review). Three other species belonging to the same family, *Potomida littoralis* (Cuvier, 1798), *Amblema plicata* (Say, 1817) (both from the subfamily Ambleminae) and *Anodonta cygnea* (Linnaeus, 1758) (subfamily Anodontinae), were also included in the analyses. *Margaritifera auricularia* (Spengler, 1793), representing the family Margaritiferidae, was used as an outgroup.

Analyses were performed on both genes together and separately for each gene. A partition homogeneity test (implemented in PAUP* as ILD test: Mickevich & Farris, 1981; Farris et al.,

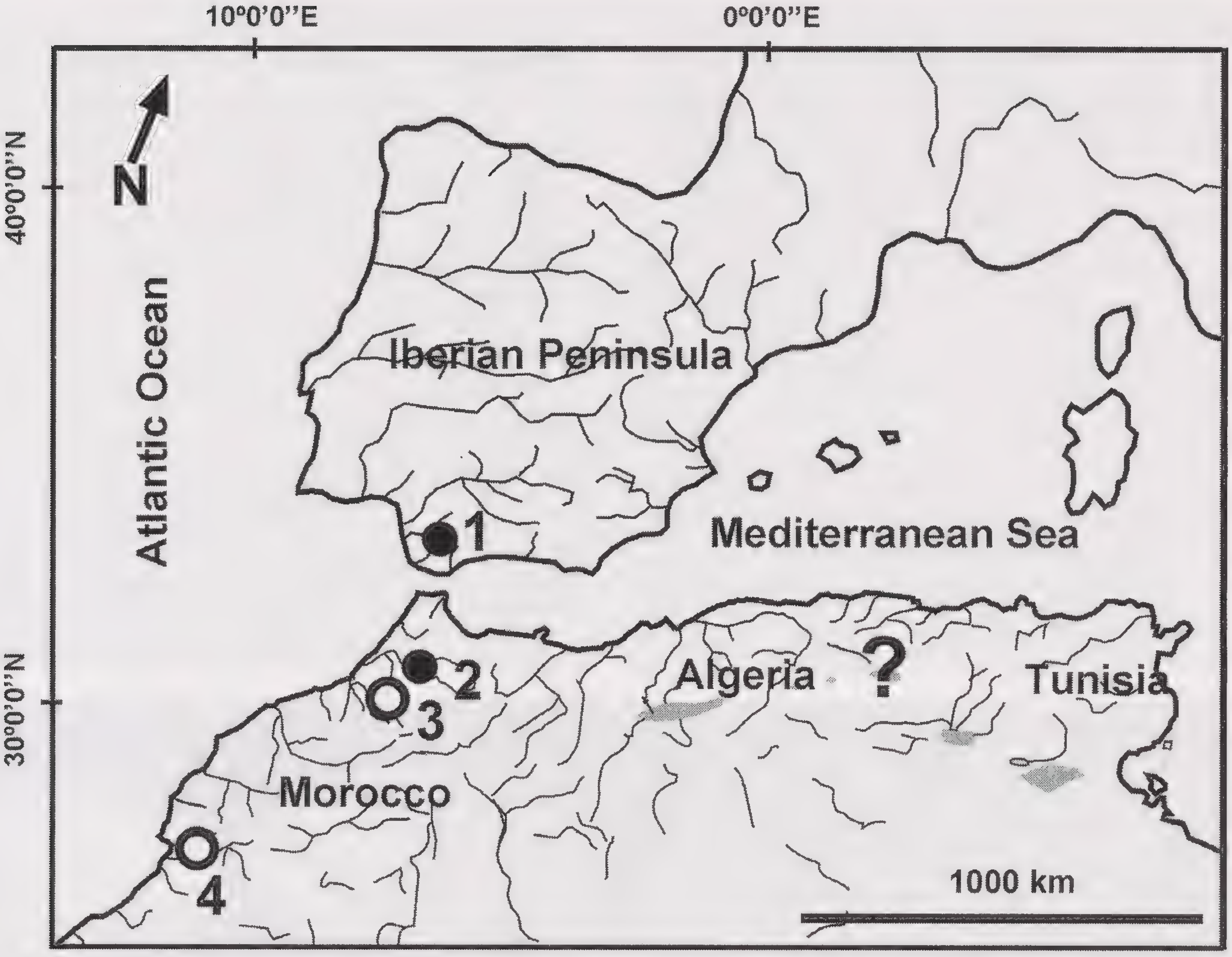

FIG. 1. Map showing the distribution of *Unio gibbus*. Black points indicate sites where live specimens have been recently discovered - 1: Barbate River; 2: Beth River; 3: Tiflet River [*Unio tifleticus* Pallary, 1923]; 4: Sous River [*Unio* (*Limniun*) *foucauldiana* Pallary, 1936].

1994) was used to assess congruence among data for the two genes. Phylogenetic analyses (maximum parsimony – MP, maximum likelihood – ML, and neighbour-joining – NJ) were conducted using PAUP* 4.0b10 (Swofford, 2001). Equal-weighted parsimony analyses were performed through heuristic searches with TBR branch swapping and ten random additions.

The appropriate model and starting parameters for ML and NJ analyses were chosen for each of the datasets using the Akaike Information Criterion implemented in Model Test 3.06 (Posada & Crandall, 1998). Maximum likelihood analysis was performed by heuristic searches, and the robustness of the topologies was assessed by bootstrap replications (1,000) (Felsenstein, 1985).

Bayesian analyses using MrBayes 3.1.1 (Huelsenbeck, 2000; Huelsenbeck & Ronquist, 2001) were conducted for each dataset, each gene separately and both together. The Bayesian analysis using one cold and three incrementally heated chains started from a random tree. The models of nucleotide evolution were estimated following a model with rate classes, unequal base frequencies and the "invgamma" option, for each gene separately and considering each codon position in the case of the COI gene. The Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analysis was run for 5,000,000 generations, sampling each 100 generations. Burn-in was 10%, after which likelihood values were stable. Majority rule consensus trees were constructed using PAUP.

RESULTS

Unio gibbus Spengler, 1793
Unio gibbus Spengler, 1793: 64.
Unio turdetanus Drouet, 1893: 66, 67; pl. 1, fig. 4.
Unio tifleticus Pallary, 1923: 78. Figured in Pallary, 1927: pl. 7, figs. 1, 2.
Unio (*Limniun*) *foucauldiana* Pallary, 1936: 63, 64; pl. 4, fig. 2.

Holotype: Zoological Museum of the University of Copenhagen, Denmark. ZMUC BIV-434. Illustrated by Haas (1913) and Knudsen et al. (2003). Type locality: "Tranquebar", India, probably in error.

Distribution (Fig. 1): Atlantic rivers of south Iberia and Morocco. Live specimens of *U. gibbus* have been recently found in two rivers only: Barbate River, Cádiz, southwest Spain, and two localities in the Beth River, Sebou basin, northwest Morocco. In the Spanish river, the species shares its habitat with *Potomida littoralis*, *Unio* cf. *pictorum* and *Anodonta* cf. *anatina* (Linnaeus, 1758), whereas in Morocco it was found living with another species of *Unio* and with *Potomida* sp.

Pallary (1923, 1927, 1936) cited *Unio tifleticus* in the Tiflet River, Sebou basin, and *Unio* (*Limniun*) *foucauldiana* in the Sous River, south of Agadir, Morocco.

External Morphology (Figs. 2, 3): Shell swollen, oval or rounded, equivalve, inequilateral, usually elevated at posterior end. Outline, especially in juvenile shells, resembling *Potomida littoralis.* Anterior end rounded in a circular arc, posterior end rounded but dorsally elevated, forming a marked keel in ligamental area. Shell thin, light; periostracum flaking in posterior and ventral areas. Colour green (more common in Moroccan specimens) to brown, sometimes with radial bands of clearer colour. Umbones rounded, prominent, never eroded, clearer in colour that rest of shell. It is common to see the glochidium as a protoconch in the apex of the umbo (Fig. 3B). Umbo sculpture more marked

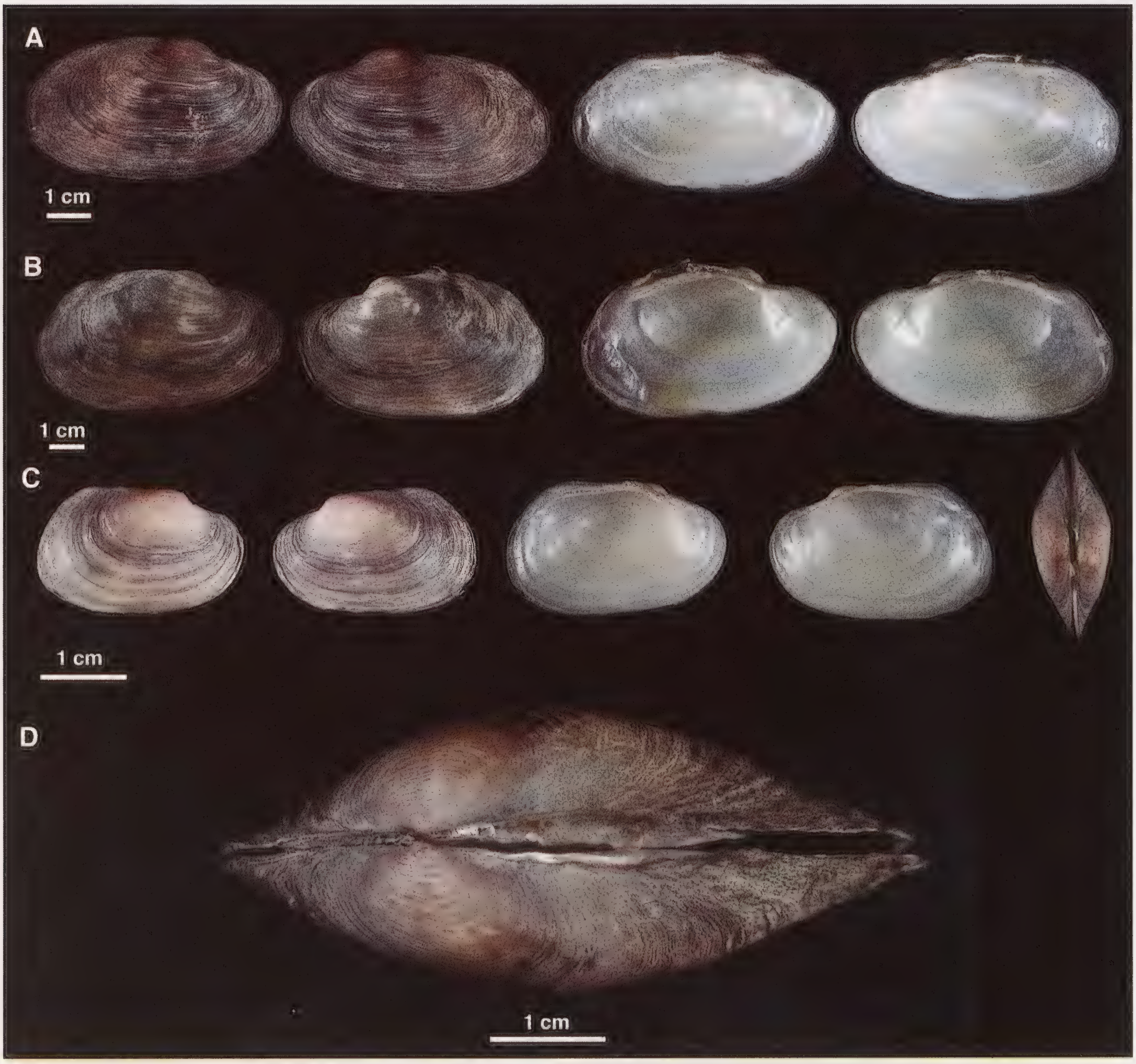

FIG. 2. *Unio gibbus* specimens from different localities. A: Barbate River, Spain; B: Beth River, Morocco; C: Juvenile specimens (Barbate River); D: Umbonal sculpture (Barbate River).

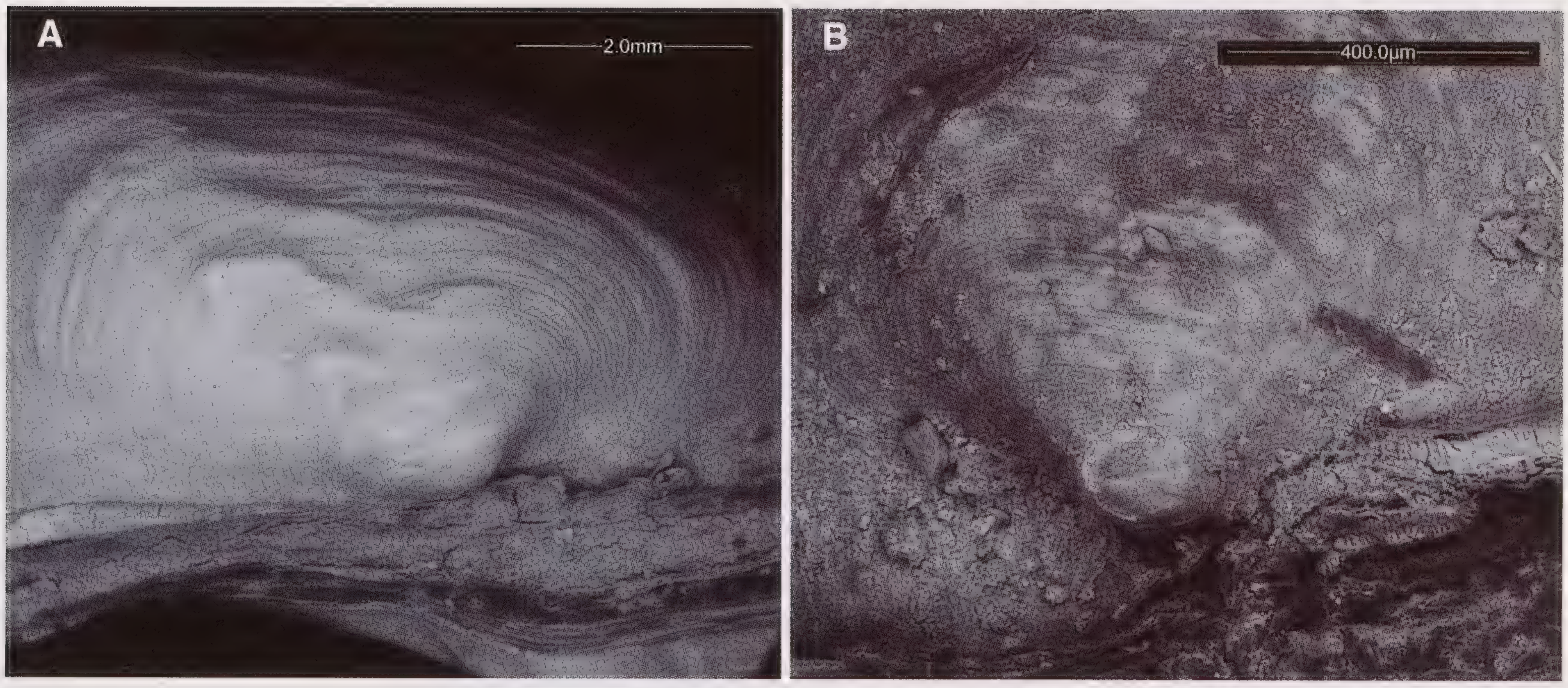

FIG. 3. *Unio gibbus* specimen. A: Detail of the umbonal sculpture; B: Remains of the glochidia present in the umbo.

in Spanish specimens, consisting of two or three rows of strong tubercula parallel to growth lines of the shell. In juvenile shells, posterior tubercula extend along a diagonal row that is absent in adult shells (Fig. 3A). Left valve with two laminar pseudo-cardinal teeth, sometimes crenulated, which may or may not be well separated; posterior tooth beaked and more elevated. Two laminar posterior lateral teeth variable in length, the lower tooth always more elevated. Right valve with pseudo-cardinal tooth generally beaked, sometimes flat, and very tall laminar lateral posterior tooth. Maximum measured shell length 85.6 mm.

Anatomy (Fig. 4): Soft parts typical of *Unio*. Papillae of the inhalant siphon generally not bifurcated; exhalant opening confined by two lateral muscular strips with external black trimming, and separated dorsally from the supra-anal aperture by a bridge shorter than the supra-anal aperture.

Life Cycle: In the Barbate River, Spain, females carried embryos in their gills at the end of February 2006, whereas in the Beth River, Morocco, ripe glochidia were found in specimens collected in early June 2007. In the Spanish specimens, the entire internal cavity of the external demibranchs (homogeneity) acted as a marsupium (ectobranchy) (Fig. 5A), but in the Moroccan population the four gills were occasionally filled with glochidia (occasional tetrageny) (Fig. 5B, C). Glochidia rounded triangular (Fig. 6), intermediate between glochidia shapes in *Unio* and *Potomida*. Border of the larval shell bears numerous small lumps enlarging toward the ventral portion where they become spicules (Fig. 6F, G, H) but never form the ventral styliform hook typical of the genus. Mean glochidial dimensions (measured by SEM): length: 209.17 µm (sd = 2.83; n = 19), height: 211 µm (sd = 3.93; n = 21), width: 67.67 µm (sd = 5.99; n = 3).

The phylogenetic relationships inferred from our data (GenBank accession numbers: EU735753 to EU735781) point to the independence of *U. gibbus* as an evolutionary unit (Fig. 7). In molecular terms, our results indicate a clear distinction between *U. gibbus* and the rest of the species analysed (Fig. 7). The Spanish specimens showed an absolute lack of genetic variability, with only one haplotype observed for each gene studied. The Iberian and Moroccan specimens of *U. gibbus* displayed a small amount of intraspecific divergence (0.37% for COI), and all shared the same haplotype for the 16S gene. In contrast, divergences were 30 times higher when compared to other *Unio* species – 10.24% with respect to the closest *U. tumidus* to 12.91% with respect to *U. tumidiformis* da Silva e Castro, 1885, for COI, and 6.18% to 8.89% for 16S with respect to the same species, respectively. These results are based on the analysis of 657 COI and 500 16S characters. Although support was low, especially for 16S, global phylogenetic analysis revealed *U. tumidus* as the sister species of *U. gibbus* (Fig. 7). Moreover, *U. gibbus* always

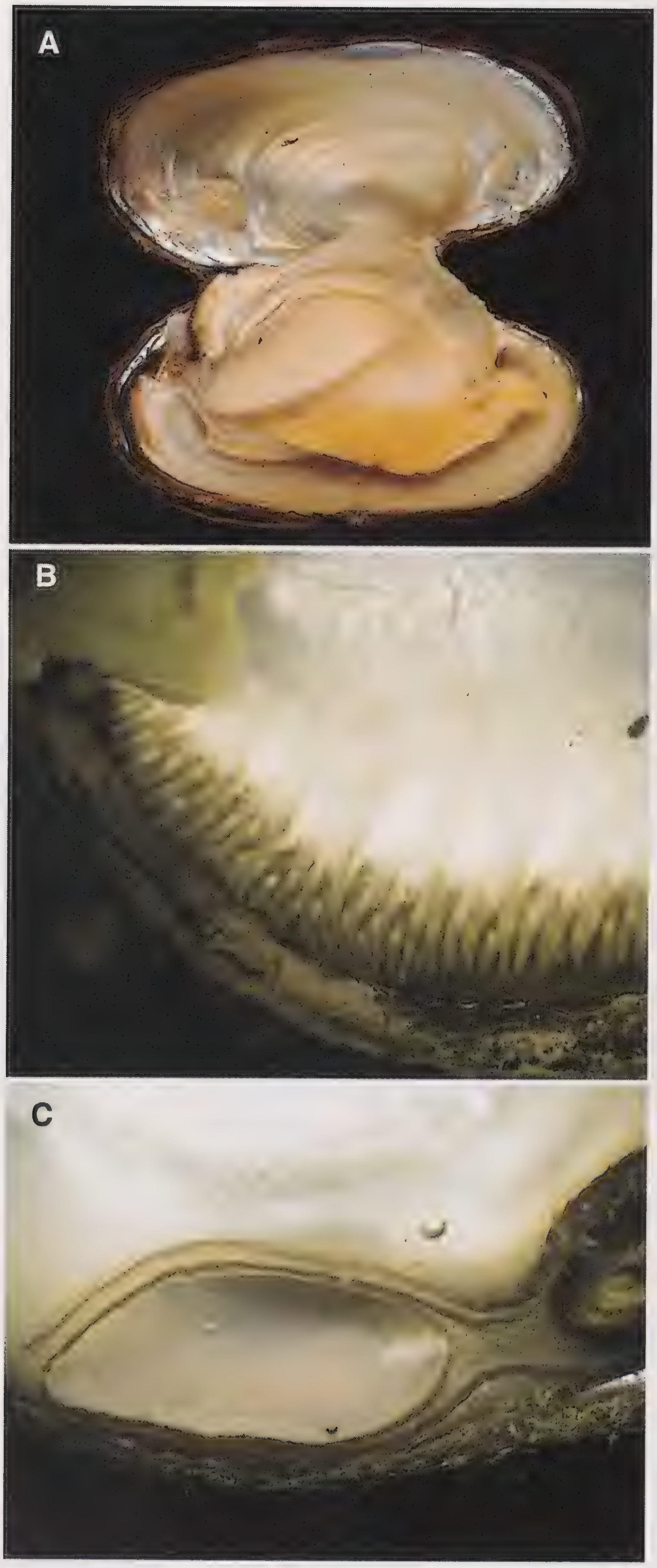

FIG. 4. Anatomy of *Unio gibbus* from the Barbate River. A: General view; B: Inhalant siphon; C: Supra anal aperture.

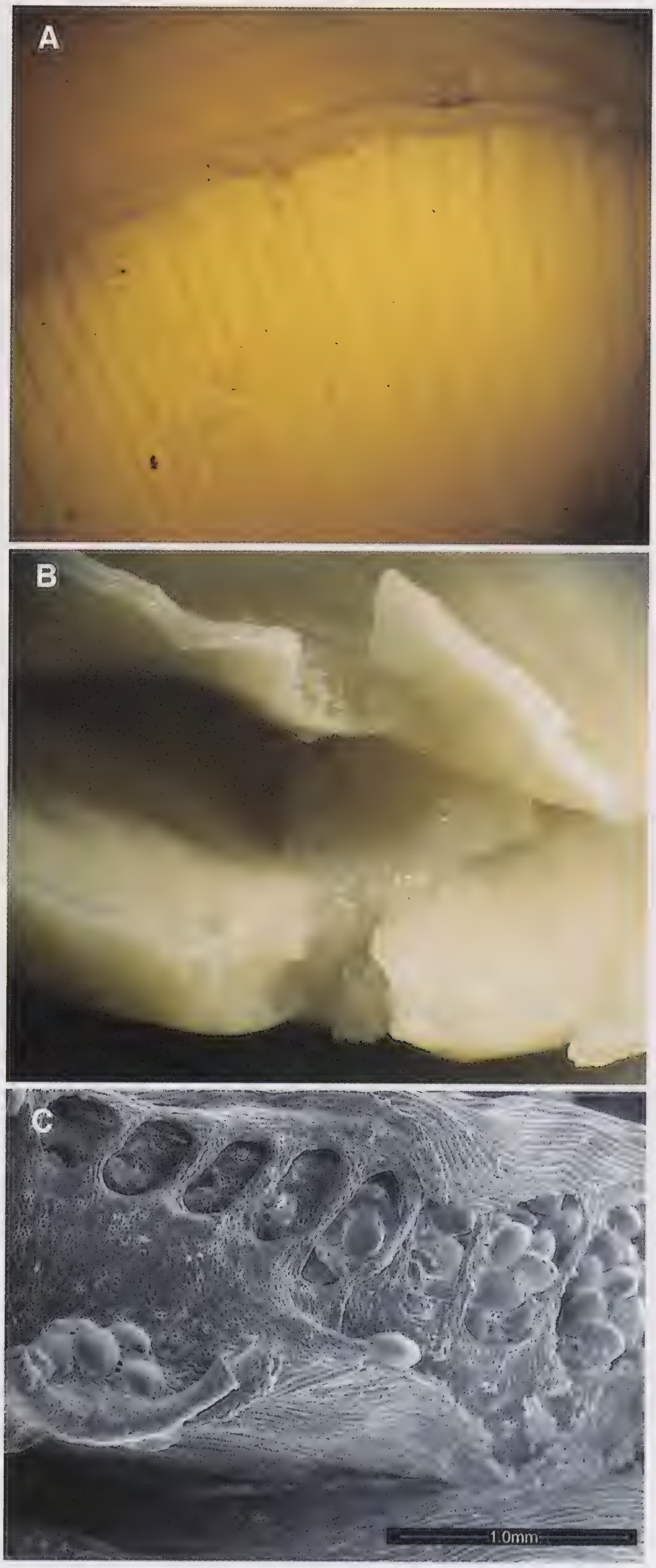

FIG. 5. *Unio gibbus* specimens. A: Gravid external gill with embryos (Barbate River); B, C: Gravid gills with glochidia (Beth River).

appeared as an independent lineage from a well-supported cluster that included the Palaearctic species *U.* cf. *pictorum*, *U. pictorum, U. mancus, U. tumidiformis*, and *U. crassus* (Fig. 7), and even the African *U. caffer* according to the COI gene (Fig. 7B). The Chinese *U. douglasiae* occupied a basal position in the 16S analyses (Fig. 7A).

Anodonta cygnea appeared as sister group of the genus *Unio* in both, COI and global analyses, but surprisingly it was included in the *Unio* cluster in the 16S dataset.

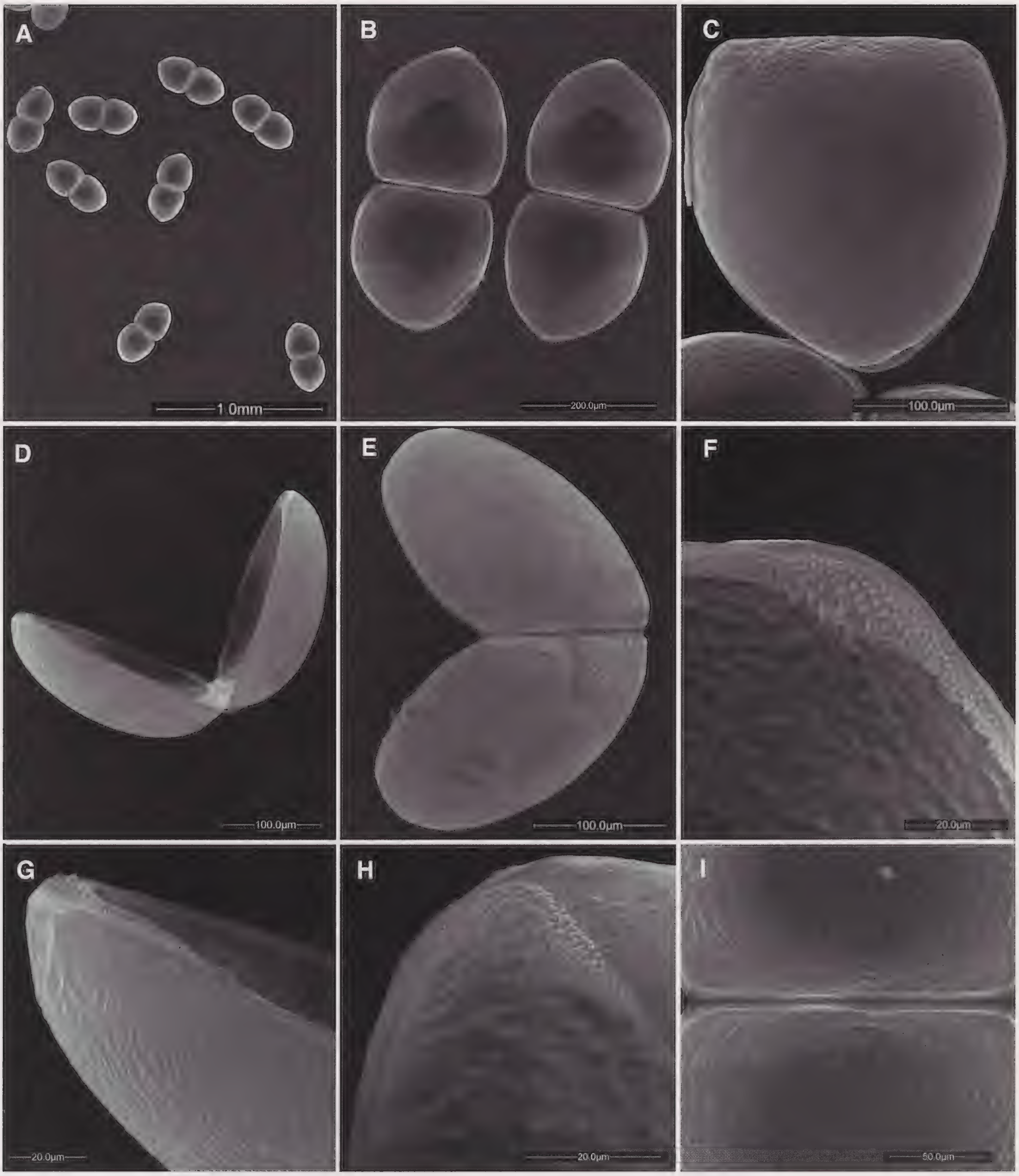

FIG. 6. Glochidium of *Unio gibbus* from the Beth River. A, B: General view; C: External view; D: Lateral view; E: Posterior view; F-H: Spicules; I: Ligament.

DISCUSSION

Simpson (1900: 861) considered *U. gibbus* as an indeterminate Oriental Unionidae. The synonymization of *Unio turdetanus* Drouet, 1893, with *Unio gibbus* Spengler, 1793, was mentioned by Haas (1913), who also suggested that the Tranquebar locality of the Spengler specimen was erroneous, and proposed that it had actually come from Spain. This is a similar error to that concerning the locality of the European *Unio auricularius* Spengler (= *Margaritifera auricularia*), which obviously did not come from East India, as indicated by Spengler (1793).

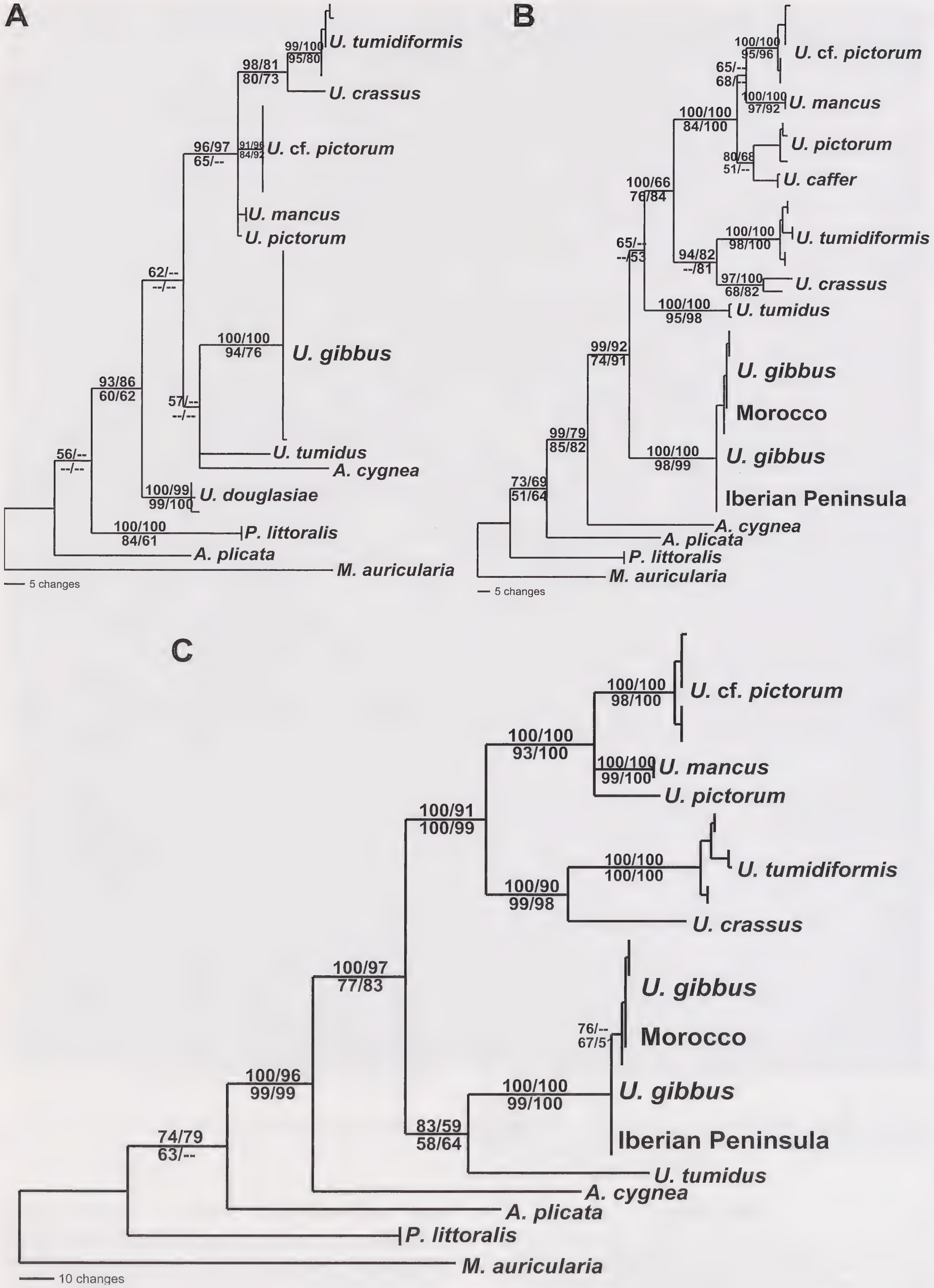

FIG. 7. Majority-rule consensus trees from Bayesian phylogenetic relationships among members of the Palaearctic genus *Unio* based on partial sequences of – A: 16S; B: COI; C: 16S + COI. Numbers above branches indicate Bayesian posterior probabilities and Maximum Parsimony bootstrap values; those below branches indicate bootstrap values for Maximum Likelihood and Neighbour-Joining treatments.

Later, Haas (1917, 1969) listed *U. turdetanus* Drouet, 1893, and *U. gibbus*, as synonyms of the southwest Atlantic Iberian *Unio pictorum delphinus* Spengler, 1793, one of the 13 taxa into which this author divided *U. pictorum*. This was probably also why Azpeitia (1933) ascribed *U. gibbus* to the same group as *U. delphinus* Spengler and *U. hispanus* Rossmässler, 1844, both synonyms of *Unio pictorum*, although he distinguished *U. gibbus* from the other species.

Unio (*Limniun*) *foucauldiana*, which we synonymize here with *U. gibbus*, was included by Daget (1998) as a synonym of the North African *U. elongatulus durieui* Deshayes, 1847, one of the 17 taxa into which Haas (1969) divided the Mediterranean species *U. elongatulus* C. Pfeiffer, 1825; nevertheless, *U.* (*Limniun*) *foucauldiana* was not included in Haas (1969). Surprisingly, *Unio tifleticus,* the other Pallary species that we consider to be a synonym of *U. gibbus,* was synonymized by both Haas (1969) and Daget (1998) with *Potomida littoralis fellmani* (Deshayes, 1848), the North African race of *P. littoralis*.

According to the characters we examined, essentially morphological, molecular, and the glochidia, *U. gibbus* differs considerably from both *U. pictorum* and *U. elongatulus*, and logically from *P. littoralis*; it is clearly a different species, probably restricted to some Atlantic Iberian and Moroccan rivers. Our molecular data also suggest that *U. gibbus* is more closely related to *U. tumidus* than to other *Unio* species.

The genetic divergences we found were similar or even greater to those observed among other unionoid species (Lydeard et al., 1996; Araujo et al., in press), but despite this clear distinction, the biogeographic implications to be inferred from the phylogenetic results are not so obvious. The current distribution of *U. gibbus* suggests differentiation in the southern Iberian Peninsula and Morocco most likely, when both areas comprised a single landmass, in the Betic-Rifian massif (Steininger & Rögl, 1984). Now, given that the Betic-Rifian massif extended as far as the current Algerian Kabilies Mountains, the possible spread of *U. gibbus* to the Mediterranean rivers of North Africa should be considered. This possibility is also suggested by the presence of *Unio* (*Limniun*) *seurati* Pallary, 1936, in Chèlif, Algeria, a very similar species to *U. gibbus* that could probably be synonymized with it.

Today's distribution of *U. gibbus* is greater in the Maghreb region than on the Iberian Peninsula. The lower molecular variation noted within the Iberian sample could reflect this reduced distribution range, indicating poor conditions in the Iberian population resulting in reduction in the effective population size and a lack of gene flow or extinction across some of its potential distribution.

It is possible that due to the long ancient history of the group, true relationships among its members or their actual biogeographical patterns are difficult or impossible to recover. Despite this, the results of the few recent studies performed on West Palaearctic unionoids (Reis et al., in review; Araujo et al., in press) are beginning to unveil a much richer diversity than currently recognized. Thus, further insight into the evolutionary history of *Unio* will have to wait until areas of such potentially high diversity areas as Turkey, Greece, and the Maghreb region are subjected to detailed investigation.

Unio gibbus has not been found in recent surveys of many Spanish South Atlantic and Mediterranean rivers, including several tributaries of the Guadalquivir basin, and the locality described by Drouet (1893) for *U. turdetanus* (= *U. gibbus*). The living population examined here was found in a short stretch of the Barbate River upstream from a large reservoir. The lack of genetic variability among these specimens, along with seasonal droughts and excessive water extractions from this river, make the Spanish populations of *U. gibbus* a main priority for invertebrate conservation measures. In Morocco, at least in the Beth River, *U. gibbus* is a very common species.

Besides the external appearance of the shell and the molecular markers rendering the species unmistakable, the most important characters that separate *U. gibbus* from other *Unio* species are tetrageny and hookless glochidia, both features informative in phylogenetic studies of the group. Interestingly, the trait of tetrageny was only detected in one Moroccan specimen, the four gills of which were filled with glochidia, the external gills being much more swollen than the internal ones. Two other gravid Moroccan specimens were carrying embryos but only in the external gills, as also occurred in the three gravid Spanish specimens. These observations suggest that the internal gills may be filled later, once the external ones are full: could the absence of embryos in the inner gills thus only be a question of time, or was the tetragenous specimen identified just an isolated case of "occasional tetrageny" with no phylogenetic implications? This question will likely be answered as we obtain more information on the reproductive strategy of other populations of this species.

Although a little broader, the size of the *U. gibbus* glochidium falls within the range cited for the already known species of the genus (Pekkarinen & Englund, 1995). However, its rounded shape, and the absence of the ventral hook make the larval shell a specific diagnostic character. It has been suggested that hooks might develop later in juvenile glochidia, such that young glochidia have no hooks, whereas older ones do (Ortmann, 1911: 301). The glochidia in our specimen were not encapsulated, so we can assume they were fully developed. Nevertheless, the idea that *U. gibbus* glochidia lack well-developed median ventral teeth should be also considered a hypothesis for future testing. As recently stated by Graf & Cummings (2006), it is yet too soon to know with certainty if tetrageny and hookless glochidia are, respectively, the plesiomorphic brooding morphology and larval type of the Unionoida. The peculiar reproductive habits discovered in *U. gibbus* fuel this rationale and reveal there are still large gaps in our knowledge of the reproductive strategies of the naiad species of the Palaearctic. Perhaps the time has come to rethink the characters presently describing the genus *Unio*.

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DIFFERENCES IN SPERMATOPHORE AVAILABILITY AMONG OCTOPODID SPECIES (CEPHALOPODA: OCTOPODA)

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ABSTRACT

Female octopuses are thought to copulate with multiple males and store sperm for months prior to spawning, a generalization that has led to hypotheses of strong sperm competition throughout the family. Their diversity, and the difficulty in observing many octopus species in the wild, force indirect tests of this generalization. Male reproductive effort, already high in these generally semelparous animals, is predicted to increase further with sperm competition. Hypothesized functional constraints on the spermatophores that males build to transfer sperm could mean that selection impacts spermatophore number more than morphology. To test whether the number of spermatophores could indicate differences in octopodid biology, the greatest number of spermatophores carried by one individual, and longest spermatophore and mantle lengths were compiled for 74 species. Differences in number were predicted to be independent of differences in specimen availability, related species were predicted to have similar counts, and counts were predicted to be largely independent of spermatophore and mantle length. The median spermatophore count was 24; nearly one-third of the species had 60 or more spermatophores. Of these, 16 were species of *Eledone, Octopus* (*Octopus*) or *Abdopus*, supporting the hypothesis that related species share similar spermatophore counts; relationships of the other species are poorly known. Spermatophores of these 24 species tended to be slightly shorter relative to mantle length than in the family as a whole. *Callistoctopus* spp. carry nine or fewer spermatophores, as do deep-sea species of *Bathypolypus*, and the Southern Ocean endemic and deep-sea octopus clade. The number of specimens did not differ between species with fewer or more than the median number. Differences in spermatophore counts among species may offer insight into male response to sperm competition and the unsuspected diversity of reproductive behavior in benthic octopods.

Key words: meristic character, sperm, male reproductive investment, *Abdopus*, *Eledone*, *Octopus* (*Octopus*), *Callistoctopus*.

INTRODUCTION

The race between sperm from two different males to fertilize an egg is termed sperm competition (Birkhead, 2000). Sperm competition may occur in any mating system in which females accept more than one male as a mate. Benthic octopuses of the Octopodidae are one such group. Data available from a very limited number of species indicate that female octopuses copulate with multiple males and store sperm for up to several months; sperm competition has therefore been suggested to be intense (Hanlon & Messenger, 1996; Birkhead, 2000). Further support for this inference derives from microsatellite analysis that found a female of *Graneledone boreopacifica* Nesis, 1982, used sperm from at least two males to fertilize her egg clutch (Voight & Feldheim, accepted) and that males of *Abdopus aculeatus* (d'Orbigny, 1834) mate-guard apparently non-selective females in the wild (Huffard et al., 2008). Female octopuses copulate with multiple males in the wild (C. Huffard, pers. comm.) and in captivity (pers. obs.).

If the intensity of sperm competition is comparable throughout the family, male reproductive investment will be uniformly high. As male paternal investment in their offspring is considered to be limited to sperm, this investment should be visible as large testis size or abundant reproductive products, as has been documented among mammals, fishes and insects (Short, 1979; Kenagy & Trombulak, 1986; Stockley et al., 1997). Among cephalopods, such differences may be apparent in spermatophores.

Several aspects of octopodid spermatophores are remarkably consistent; the length of the sperm reservoir, the sperm-carrying part of the spermatophore, correlates very tightly with total spermatophore length across the family, except in species of the genus *Eledone*, which have a uniformly longer sperm reservoir (Voight, 2001). Spermatophore length shows limited variation, other than that due to body size (Voight, 2002; Allcock et al., 2008).

To determine if male allocation to reproduction differs among octopodid species, I tested for differences in the number of spermatophores that males of different species carry. Differences found may offer a predictive framework for future behavioral studies, as they may suggest differences in mating systems. Spermatophore count will be rated as informative if differences in the number of specimens considered are not linked to differences in counts, if species that share the possession of many or of few spermatophores are closely related, and if the few species known to have unusual reproductive behaviors also have abundant spermatophores. Apparently nonsensical variation in spermatophore number would give evidence that the number of spermatophores carried is uninformative for identifying unusual reproductive biology.

Octopodid Reproductive Biology

Male octopuses package sperm into spermatophores that they transfer to females via a modified arm, the hectocotylus (Wodinsky, 2008). Most information about how cephalopod spermatophores function derives from studies of Mann et al. (1970), summarized here. The long, thin spermatophores of octopuses contain two parts, the sperm reservoir and the ejaculatory apparatus. Spermatophore fluid is characterized by high molecular weight chemicals that generate a hyperosmotic fluid; the spermatophore's outer tunic functions as a semi-permeable membrane. Once a male's internal reproductive organs release a spermatophore and it contacts seawater, water is drawn inside the spermatophore. The increased internal pressure causes the ejaculatory apparatus of the spermatophore to extrude, presumably after it has been inserted into an oviduct (females have two). In time, the extruded ejaculatory apparatus balloons out to form the spermatophoric bladder; its eventual rupture releases sperm into the female's oviduct. The available data suggest that in octopodids, except species of *Eledone* detailed below, and perhaps of *Vulcanoctopus*, sperm travels to spermathecae in the oviducal glands for storage. Insemination is thought to occur when mature eggs, released from the ovary, pass through the oviducal glands (Froesch & Marthy, 1975).

The reproductive anatomy of species of *Eledone* is nearly unique; males produce spermatophores with large sperm reservoirs (Voight, 2001) and females lack spermathecae (Perez et al., 1990). In South American species of *Eledone*, sperm penetrate ovarian eggs shortly after copulation, suggesting that the first male to copulate with a female is most likely to inseminate her eggs (Perez et al., 1990). The only known female specimen of *Vulcanoctopus hydrothermalis* González et al., 1998, also reportedly lacks spermathecae (González et al., 2008).

MATERIALS AND METHODS

Spermatophore Counts

The maximum number of spermatophores, and the longest mantle and spermatophore lengths were compiled from as many octopodid species as possible. The specimens examined are reported in Table 1, their depositories are: CAS = California Academy of Sciences; FMNH = Field Museum of Natural History; UA = University of Arizona collections. Because available specimens represented a narrow range of octopodid diversity, and not all specimens had intact spermatophores that could be both reliably measured and accurately counted, literature reports provided the second, and far more complete, data source (Table 1).

Use of maxima stresses the most comparable data (perhaps by comparing males with the lowest mating success), although it cannot assure full comparability. Different species are inevitably represented by different numbers of males that likely have attained different levels of maturity, and have had different reproductive histories. The number of spermatophores a male carries has been suggested to vary seasonally, especially if reproductive activity is seasonal, and with the male's recent sexual history (Mangold-Wirz, 1963). In octopodids, once begun, spermatophore production is thought to be continuous (Mangold, 1987), suggesting that an unmated, fully mature male will carry every spermatophore ever produced. However, as the capacity of the extensible storage (Needham's) sac is likely finite, some spermatophores seemingly would have to be shed to accommodate those newly produced. Wodinsky (2008) supported this hypothesis by documenting that males release spermatophores in the absence of any known reproductive stimuli. When recognized, anomalous spermatophore counts, due for instance to occluded male reproductive organs (Wodinsky, 2008), were ignored. For example, Mann et al. (1970) reported a male of *Enteroctopus dofleini* (Wülker, 1910) had 32 spermatophores in its storage sac due to an occlusion; because this individual was considered aberrant, Pickford's (1964) report of 12 spermatophores was cited here as the maximum count for the species. Silva et al.'s (2002) plot of male size and spermatophore count among 2,333 males of *Octopus vulgaris* Cuvier, 1797, found the two showed no relationship; the two highest counts were 276 and near 190. The latter was selected as the more conservative and consistent with the report of 125 spermatophores for the species by Mangold-Wirz (1963). Although one of the 25 known male specimens of *Vulcanoctopus hydrothermalis* (González et al., 2002; pers. obs.) carried 114 spermatophores, the next highest value, 74, was cited here.

In total, data were compiled from 74 species, which are assigned to 13 groups (Table 1). Nine groups, *Abdopus*, *Amphioctopus*, *Bathypolypus*, the *Benthoctopus* clade as defined by Strugnell et al. (accepted), *Callistoctopus*, *Eledone*, *Octopus* (*Octopus*) sensu Norman and Hochberg (2005a), *Scaeurgus* and the Southern Ocean endemic and deep-sea octopuses, defined as a clade by Strugnell et al. (in press; unpublished data), can be argued to be monophyletic. Our poor understanding of octopodid phylogeny (Voight, 1997) precludes assigning the remaining species to comparable groups. For instance, Norman & Hochberg's (2005a) restricted definition of *Octopus* left many species unplaced; they are here considered *Octopus* implicitly *sensu lato*. To simplify the presentation of the remaining 26 species, four artificial groups are created. These artificial groups are not based on hypothesized relationships, but serve only as a convenience to organize these species. One such group contains long-armed octopuses, a second contains species apparently restricted to shelf and slope depths from 100 to 600 m, the third includes species with maximum mantle lengths under 35 mm, analogous to the Pygmy octopuses of Norman & Sweeney (1997), and the fourth contains the remaining species.

Data Analyses

The median rather than the mean of number of spermatophores carried by the 74 species was used here because the data are not normally distributed. Species with at least two and half times the median number of spermatophores are arbitrarily defined as having abundant spermatophores. To test whether specimen availability affected the species' estimated number of spermatophores, the number of specimens representing species with more than the median number of spermatophores was compared to the number of specimens representing species with fewer than the median number of spermatophores using a Kolmogorov-Smirnov (K-S) test.

To describe the relationship between spermatophore count and body size, the maximum number of spermatophores for each species was plotted versus the longest mantle length reported for that species. A log-normal plot was generated to better illustrate the wide size range of species considered. Mantle lengths of males of *Callistoctopus aspilosomatus* (Norman, 1993a) that are "elongate" were not considered to ensure comparability.

To test whether abundant spermatophores tend to be comparatively short, log-transformed longest spermatophore length for each species was plotted versus the log of the longest mantle length for that species. Log-transformation equalizes measurement error over the wide size range considered, minimizes any error introduced by using back-calculated measurements or investigator bias, and minimizes the effect of outliers (Bookstein et al., 1985; Strauss, 1985). A regression line was generated based on all points. The distribution of points representing species with abundant spermatophores relative to the regression line (i.e., above, below) was compared to the expected 50:50 distribution about the line and tested with a G-test.

Behavioral Observations

Limited behavioral observations suggest differences exist in the potential for sperm competition among octopodids. Huffard et al. (2008) report mate guarding and sneaker males in *Abdopus aculeatus.* Multiple males have been observed in the wild attempting to copulate with lone females of *Octopus (O.) cyanea* Gray, 1849; *O. (O.) bimaculatus* Verrill, 1883 (Hanlon & Messenger, 1996; Tsuchiya & Uzu,

TABLE 1. Reported by group are each species included, its taxonomic authority, highest spermatophore count, number of male specimens considered, the longest mantle and spermatophore lengths in mm (ML; SpL) and the data source.

Taxon, Authority	Count	n	ML	SpL	Data source
Abdopus Norman & Finn, 2001; n = 9					
A. horridus (d'Orbigny, 1826)	70	10	30	12.8	Norman & Finn (2001)
A. aculeatus (d'Orbigny, 1834)	157	3	58	21	Norman & Finn (2001)
*A. abaculus (*Norman & Sweeney, 1997)	126	5	33	9.2	Norman & Sweeney (1997)
*A. capricornicus (*Norman & Finn, 2001)	135	3	43	13.5	Norman & Finn (2001)
A. undulatus Huffard, 2007	91	3	33.5	9.3	Huffard (2007)
A. sp. 2 (Philippines, Babuyan, Lubang Islands)	117	4	30	12.5	Norman & Finn (2001)
A. sp. A Gulf of Thailand	152	1	43	28	CAS 077999
A. sp. B Gulf of Thailand	157	1	58	15.6	CAS 078002
A. sp of Ward Guam	160	8	35	12	Norman & Finn (2001)
Amphioctopus Fischer, 1882; n = 7					
A. aegina (Gray, 1849)	18	4	62	27	Norman & Sweeney (1997)
A. arenicola Huffard & Hochberg, 2005	11	3	51	37	Huffard & Hochberg (2005)
A. burryi (Voss, 1950)	11	1	47	23.6	Voss (1951)
A. exannulatus (Norman, 1993b)	8	1	50	62	Norman (1993b)
*A. mototi (*Norman, 1993b)	9	3	70	46.5	Norman (1993b)
A. polyzenia (Gray, 1849)	10	1	26	9	Norman (1993b)
A. sp. 1 Philippines, Visayan Sea	5	1	45	25	Norman & Sweeney (1997)
Bathypolypus Grimpe, 1921; n = 3					
B. arcticus (Prosch, 1849)	6	6	65	64	Muus (2002)
B. bairdii (Verrill, 1873)	6	6	90	72	Muus (2002)
B. sponsalis (Fischer & Fischer, 1892)	8	> 200	70	34	Mangold-Wirz (1963)
Benthoctopus clade (Strugnell et al., accepted); n = 8					
Enteroctopus dofleini (Wülker, 1910)	12	4	330	1130	Pickford (1964)
E. magnificus (Villanueva et al., 1991)	8	41	362	870	Villanueva et al. (1991)
Benthoctopus johnsonianus Allcock et al., 2006	19	1	113	104	Allcock et al. (2006)
B. karubar Norman et al., 1997	26	1	60	41	Norman et al. (1997)
B. longibrachus Ibáñez et al., 2006	31	12	115	70	Ibáñez et al. (2006)
B. normani (Massy, 1907)	25	9	100	119	Allcock et al. (2006)
Benthoctopus n. sp.	24	2	34	25.8	FMNH 278309
Vulcanoctopus hydrothermalis González et al., 1998	74	18	56.4	54	González et al. (2002)
Callistoctopus Taki, 1964; n = 6					
C. nocturnus (Norman & Sweeney, 1997)	1	1	60	28.2	Norman & Sweeney (1997)
C. alpheus (Norman, 1993a)	3	2	74	68.5	Norman (1993a)
C. aspilosomatis (Norman, 1993a)	2	3	45	39	Norman (1993a)
C. dierythraeus (Norman, 1993a)	4	1	135	102	Norman (1993a)

(continues)

(continued)

Taxon, Authority	Count	n	ML	SpL	Data source
Callistoctopus graptus (Norman, 1993a)	5	1	190	135	Norman (1993a)
C. ornatus (Gray, 1849)	9	2	104	51	Norman & Sweeney (1997)
Eledone Leach, 1817; n = 4					
E. cirrhosa (Lamarck, 1789)	290	50	110	54	Boyle & Knobloch (1984)
E. gaucha Haimovici, 1988	92	40	41	12.1	Haimovici (1988)
E. massyae Voss, 1964	60	111	80	30	Perez & Haimovici (1991)
E. moschata (Lamarck, 1789)	172	41	150	18.3	Akyol et al. (2007)
Octopus (Octopus) Cuvier, 1797; n = 3					
O. (O.) vulgaris Cuvier, 1797	190	2333	250	65	Silva et al. (2002)
O. (O.) cyanea Gray, 1849	> 300	ca. 15	160	48	Norman & Sweeney (1997)
O. (O.) mimus Gould, 1852	65	5	155	58	Guerra et al. (1999)
Southern Ocean endemic and deep-sea *Octopus* clade (Strugnell et al., in press; A. L. Allcock, pers. comm.); n = 4					
Graneledone boreopacifica Nesis, 1982	5	15	129	131	FMNH 278304
G. gonzalezi Guerra et al., 2000	5	5	84	100	Guerra et al. (2000)
Pareledone felix Allcock et al., 2007	6	8	42.4	70	Allcock et al. (2007)
Vosseledone charrua Palacio, 1978	3	1	53	56	Palacio (1987)
Scaeurgus Troschel, 1857; n = 4					
S. unicirrhus (Della Chiaje, 1839)	12	23	56	51	Mangold-Wirz (1963)
S. tuber Norman et al., 2005	8	5	17.8	12	Norman et al. (2005)
S. juneau Norman et al., 2005	8	4	35.7	36	Norman et al. (2005)
S. neisis Norman et al., 2005	7	3	47.3	30	Norman et al. (2005)
Long-armed octopuses; n = 3					
Ameloctopus litoralis Norman, 1992	70	2	30	6.4	Norman (1992)
Thaumoctopus mimicus Norman & Hochberg, 2005b	26	2	42.1	17	Norman & Hockberg (2005b)
Wunderpus photogenicus Hochberg et al., 2006	31	2	22	10.4	Hochberg et al. (2006)
Pygmy species (ML< 35 mm); n = 9					
Octopus bocki Adam, 1941	114	2	25	7	Norman & Sweeney (1997)
O. fitchi Berry, 1953	12	4	29	19	UA specimens
Octopus gorgonus Huffard, 2007	55	4	24	7.7	Huffard (2007)
O. humilis Huffard, 2007	30	1	26.4	17.5	Huffard (2007)
O. mariles Huffard, 2007	48	3	25	26.7	Huffard (2007)
O. pumilus Norman & Sweeney, 1997	110	5	31	7.8	Norman & Sweeney (1997)
O. wolfi (Wülker, 1913)	30	2	15	8.7	Norman & Sweeney (1997)
O. sp. 3 Philippines, Southern Negros, NW Mindanao Islands	68	2	21	9	Norman & Sweeney (1997)
O. sp. 5 Philippines, Cuyo Islands, S. negros and NW Mindanao; Cocos Keeling Islands	24	1	24	8.3	Norman & Sweeney (1997)
Miscellaneous species; n = 8					
Cistopus indicus (Rapp, 1835)	13	6	86	30	Norman & Sweeney (1997)

(continues)

(continued)

Taxon, Authority	Count	n	ML	SpL	Data source
Hapalochlaena fasciata (Hoyle, 1886)	50	1	45	16	Tranter & Augustine (1973)
Macroctopus maorum (Hutton, 1880)	13	46	310	228	Grubert & Wadley (2000)
Octopus filosus Howell, 1867	90	38	37	10	Alvina (1965)
O. laqueus Kaneko & Kubodera, 2005	69	7	40.6	22	Kaneko & Kubodera (2005)
O. cf. vitiensis Hoyle, 1885	106	2	42	21.5	Norman & Sweeney (1997)
O. sp. 4 Philippines, Batan Islands	12	1	42	16.7	Norman & Sweeney (1997)
Paroctopus digueti (Perrier & Rochebrune, 1894)	25	15	42	22	UA specimens
Shelf and slope species (100 to 600 m depth); n = 6					
Galeoctopus lateralis Norman et al., 2004	8	9	30	33	Norman et al. (2004)
Octopus micros Norman, 2001	4	2	25	22.8	Norman (2001)
O. palari (Lu & Stranks, 1991)	1	1	54	25.1	Norman et al. (1997)
O. pyrum Norman et al., 1997	3	3	35	26	Norman et al. (1997)
O. salutii Verany, 1839	45	192	110	74	Mangold-Wirz (1963)
Pteroctopus tetracirrhus (Della Chiaje, 1830)	5	> 200	107	84	Mangold-Wirz (1963)

1997); *Vulcanoctopus hydrothermalis* (Rocha et al., 2002); and *O. kaurna* Stranks, 1990 (Norman, 2000). These species are predicted to have abundant spermatophores.

RESULTS

The maximum number of spermatophores carried by an individual male in the 74 octopodid species considered ranges from 1 to over 300 (Table 1); the median maximum number of spermatophores is 24. The number of specimens considered does not significantly differ between species with more than and with fewer than the median number of spermatophores ($D = 0.1622$, $P = 0.676$); the low value of the median cannot be attributed to sampling artifact.

The relationship between the number of spermatophores and log mantle length (Fig. 1) is not significant ($r = 0.072$). The relationship between number of spermatophores and log spermatophore length is statistically significant ($p < 0.05$, df = 70), but the predictive value of the relationship ($r^2 = 0.064$) is very low.

The 24 species with abundant (60 or more) spermatophores are not randomly distributed through the family (Fig. 1; Table 1). Sixteen of the 24 species are in one of three groups: *Abdopus, Eledone, Octopus* (*Octopus*). One member of the *Benthoctopus* clade, *V. hydrothermalis,* has abundant spermatophores, but other than *Enteroctopus*, the basal member of the group (Allcock et al., 2006), the remainder carry about the median number. Seven species with abundant spermatophores, *Ameloctopus litoralis* Norman, 1992; *O. filosus* Howell, 1867; *O. pumilus* Norman & Sweeney, 1997; *O. cf. vitiensis* Hoyle, 1885; *O. bocki* Adam, 1941; *O.* sp. 3 of Norman & Sweeney (1997); and *O. laqueus* Kaneko & Kubodera, 2005, remain very poorly known, both in terms of systematic relationships and general biology.

Nearly a quarter of the 24 species carrying nine or fewer spermatophores are members of *Callistoctopus*; eight are species known from shelf and slope depths (Table 1); four are members of the Southern Ocean endemic and deep-sea clade (Strugnell et al., in press, A. L. Allcock pers. comm.), and three are species of *Bathypolypus* and of *Amphioctopus. Octopus salutii* Verany, 1839; *Scaeurgus unichirrus* (Della Chiaje, 1839); and species of *Benthoctopus* are exceptional among deeper-water species in carrying from 12 to 45 spermatophores (Table 1).

Maximum spermatophore length generally increases with maximum mantle length among the 74 octopodid species considered ($r^2 = 0.716$; Fig. 2). In species with abundant spermatophores, spermatophore lengths are significantly more likely to fall low on plots of log

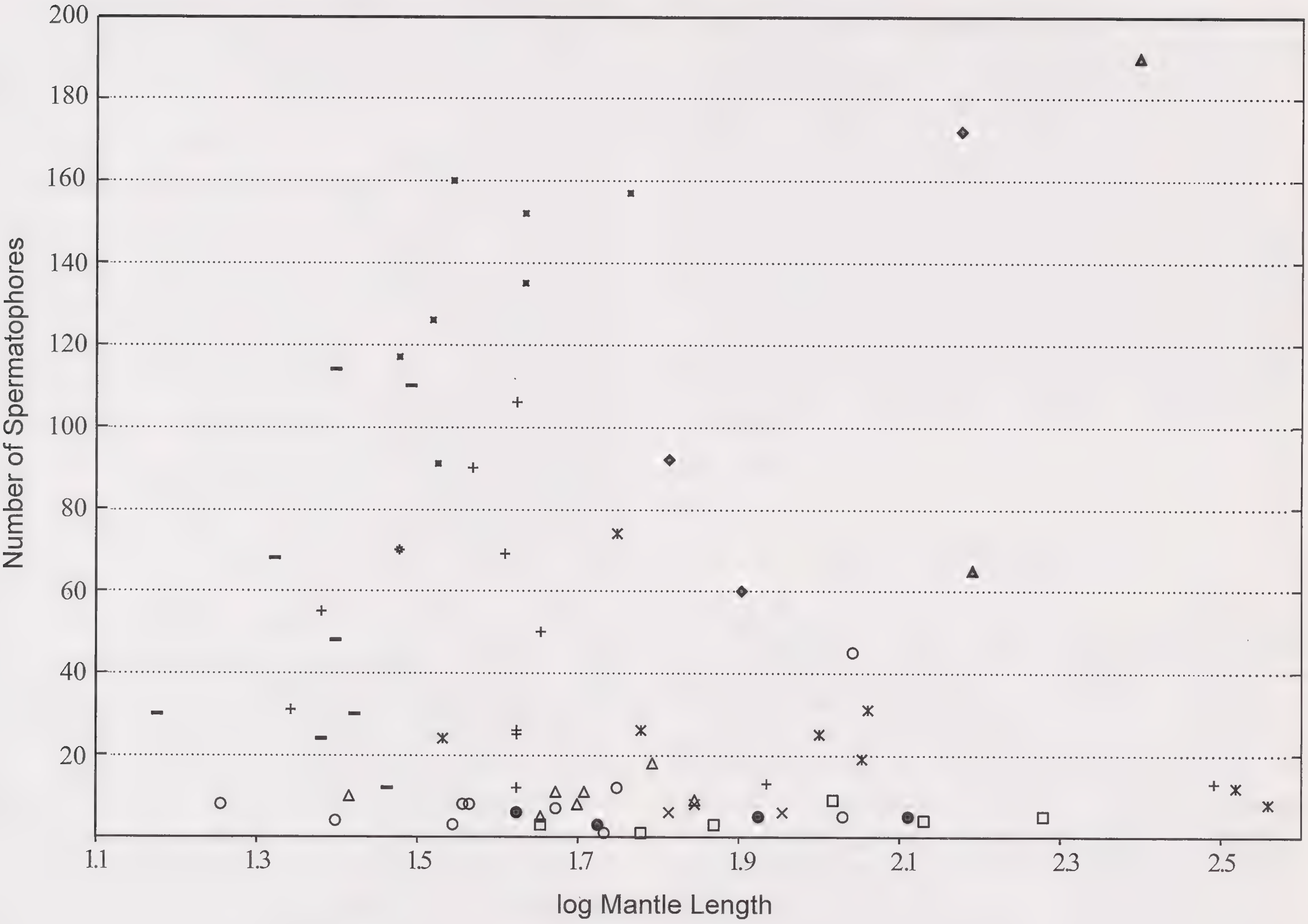

FIG. 1. Plotted is the maximum number of spermatophores carried by an individual male versus the longest log-transformed mantle length for 72 of the 74 species considered. Excluded are *Octopus cyanea* in which the count exceeds 300 spermatophores (Norman & Sweeney, 1997) and *Eledone cirrhosa* in which the count reaches 290 (Boyle & Knobloch, 1984). Groups with abundant spermatophores are *Eledone* (solid diamond), *Abdopus* (solid square), *O. (Octopus)* (solid triangle). Groups from low temperatures are *Bathypolypus* (X), the *Benthoctopus* clade (X with a vertical line), South Ocean endemic and Deep-sea clade (solid circle) and Shelf and slope species, including *Scaeurgus* (open circle). Species of *Amphioctopus* are represented by open triangles, those of *Callistoctopus* by open squares, and the artificial groups of pygmy species by bars and long-armed and miscellaneous species by +.

spermatophore length versus log mantle length (Fig. 2) than in species with fewer spermatophores ($G = 6.2$; $P < 0.025$). *Vulcanoctopus hydrothermalis*, a robust specimen of *Abdopus* sp. A (CAS 077999), *O. laqueus*, and *O.* cf. *vitiensis* are exceptional in having abundant spermatophores that are at least as long as predicted by the regression line calculated for all 74 species (Fig. 2).

DISCUSSION

Sixteen of the 24 species identified here as having abundant spermatophores, arbitrarily defined as 60, or two and a half times the median, are members of three groups, *Abdopus*, *Eledone* and *Octopus* (*Octopus*). A quarter of the species with nine or fewer spermatophores are members of the genus *Callistoctopus*. Others are all species of *Bathypolypus* included and the Southern Ocean endemic and deep-sea clade containing *Graneledone*, *Pareledone*, and *Vosseledone* (Strugnell et al., in press; J. Strugnell & L. Allcock, pers. comm.). The hypothesis that related species share spermatophore counts is supported, although seven other species with abundant spermatophores – *Ameloctopus litoralis*, three of eight pygmy species, and three miscellaneous species – can currently only be assigned to groups of convenience. Given that spermatophore counts are not impacted by differences in the number of specimens considered, are abundant spermatophores associated with unusual reproductive patterns?

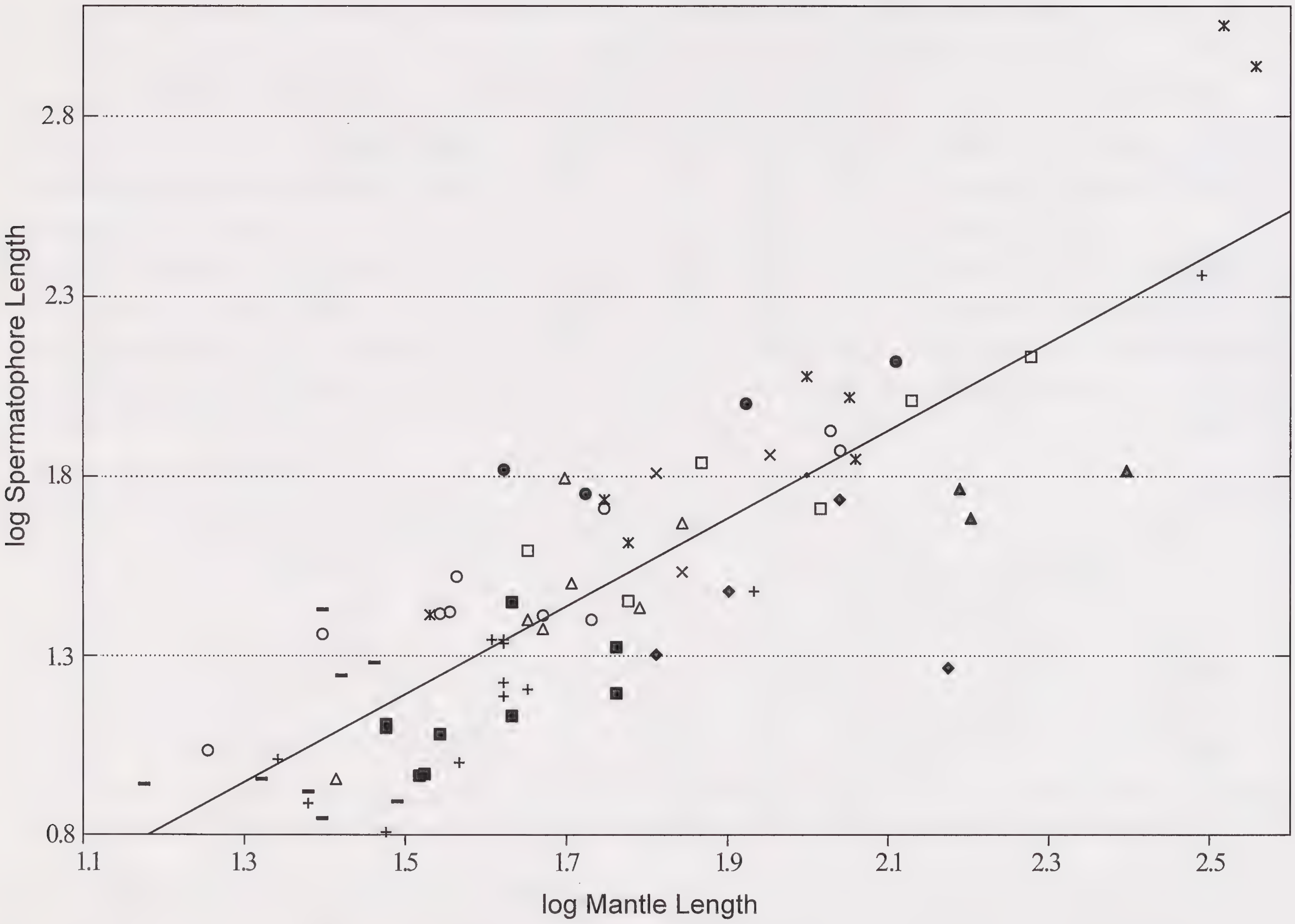

FIG. 2. Plotted are log-transformed spermatophore length versus log-transformed mantle length for the 74 species considered here. The line is the relationship of spermatophore and mantle length for all species considered (y = 1.22x – 0.646). Groups with abundant spermatophores are *Eledone* (solid diamond), *Abdopus* (solid square), *O. (Octopus)* (solid triangle). Groups from low temperatures are *Bathypolypus* (X), the *Benthoctopus* clade (X with a vertical line), South Ocean endemic and Deep-sea clade (solid circle) and Shelf and slope species including *Scaeurgus* (open circle). Species of *Amphioctopus* are represented by open triangles, those of *Callistoctopus* by open squares, and the artificial groups of pygmy species by bars, and long-armed and miscellaneous species by +. The two exceptionally large spermatophores are those of *Enteroctopus* species.

The presence of abundant spermatophores in *Eledone* is consistent with their known reproductive biology. As discussed above, males of *Eledone* that copulate with the greatest number of virgin females likely sire the most offspring (Perez et al., 1990). Having abundant spermatophores may be vitally important to increasing male fitness, especially in dense, reproductively synchronized populations of *Eledone*. The only known female specimen of *Vulcanoctopus hydrothermalis* also reportedly lacks spermathecae (González et al., 2008), and multiple males have been reported to grapple with a lone female (Rocha et al., 2002). Both the lack of spermathecae and multiple mates are predicted to select for an increase in the number of spermatophores available.

As summarized above, among species in which eggs are assumed to be inseminated as they pass through the oviduct at spawning, at least two males have been reported attempting to copulate with a lone female of *A. aculeatus*, *O. (O.) cyanea*, and *O. (O.) bimaculatus*, all species with abundant spermatophores, as predicted by the observed mating. Males also appear to grapple over females in *O. kaurna* (Norman, 2000); spermatophores in that species are predicted to be abundant.

Also deserving attention are species that appear to carry very few spermatophores, notably those of *Callistoctopus*. All six members of this genus included here carry nine or fewer spermatophores, however, each of these species is represented by fewer than ten specimens (Table 1). Although too few specimens are available for robust statistical tests, among the 30 other species that are represented by so few males, only 26.6% carry so few spermatophores. The few spermatophores carried by males of *Callistoctopus* may therefore reflect a real difference in the reproductive biology of the group.

Deep-sea octopuses are considered to have a few, large spermatophores as an adaptation to the deep sea (Voss, 1988), as shown by *Bathypolypus* and the Southern Ocean endemic and deep-sea clade of Strugnell et al. (in press). Species of *Benthoctopus* included here (Table 1), however, carry at least the median number of spermatophores for the family as a whole. Spermatophores of octopuses that live in cold temperatures are larger than predicted by mantle length (Fig. 2), perhaps because low temperatures increase cell and egg size, as occurs in ectotherms of diverse phyla (Van Voorhies, 1996). Although the pattern is yet to be experimentally demonstrated in cephalopods, both the squid *Loligo gahi* Orbigny, 1835 (Arkhipkin et al., 2000), and the octopodid *Eledone cirrhosa* (Lamarck, 1789) (Boyle et al., 1988) produce larger eggs and spermatophores in cold water than do conspecifics in warmer waters, supporting this hypothesis.

Spermatophore counts appear to be independent of differences in specimen availability and to vary within narrow limits among closely related species. A necessary simplifying assumption of this study is that all spermatophores are created equal, regardless of apparent differences in sperm mass thickness or coiling. Our knowledge of these differences, and of the chemistry of spermatophore fluid, must be increased to test the generality of that assumption.

Although it has received minimal attention, spermatophore width is a very important variable. A key determinant of volume, width may also affect the rate of the spermatophore reaction. If abundant spermatophores are not only shorter, but thinner, as limited data suggest, their greater surface area would accelerate the movement of water into the spermatophore and speed the spermatophoric reaction. Faster spermatophore reactions might be important where male competition is intense or matings are brief or occur while foraging. When a spermatophore is inserted into an oviduct, and presumably held by the muscular oviduct, the outer casing can dangle free. Removing the spermatophore before the spermatophoric reaction is complete, that is before the sperm bladder is free of the outer casing, would result in loss of the sperm. Faster reactions, as maybe achieved with thinner spermatophores, might minimize this risk. That males attempt to remove partially everted spermatophores seems more likely than suggestions (Norman, 2000; Norman et al., 2004) that males scrape sperm from the female's oviducts with their ligula, or that in *Galeoctopus lateralis* Norman et al., 2004, use their ligula to rupture the "outer casings of the sperm bulbs of previous suitors".

Regardless, octopodid reproductive biology likely contains far greater diversity than has been expected. Behavioral observations are required to test whether the species with unusual spermatophore counts have unusual reproductive behaviors, but count data appear to offer a framework on which predictions can be made.

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RESEARCH NOTES

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SEQUENTIAL PATHWAYS IN THE MATING BEHAVIOR OF THE APPLE SNAIL *POMACEA CANALICULATA* (CAENOGASTROPODA: AMPULLARIIDAE)

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INTRODUCTION

Pomacea canaliculata is the most studied ampullarid in many aspects of reproductive biology, such as environmental factors triggering reproduction (Albrecht et al., 1999, 2005), functional properties of eggs (Heras et al., 2007), reproductive strategy and effort (Estebenet & Cazzaniga, 1993; Estoy et al., 2002b), and size and age at which maturity is reached (Estoy et al., 2002a; Tamburi & Martín, 2009). Surprisingly, though, the current knowledge on the details of mating behavior is scarce.

Males of *Pomacea canaliculata* have a complex copulatory apparatus of pallial origin. It comprises a bulky and muscular penis sheath with a deep channel on its inner face, along which a slender penis runs during copula (Andrews, 1964; Gamarra-Luques et al., 2006). Knowledge on mating behavior of *P. canaliculata* comes mostly from non-systematic observations: matings are long lasting (up to 20 h), occur under water at any time of the day or night, and females can crawl freely but the males cannot and therefore depend on the females' movements to reach the water surface and ventilate their lungs (Andrews, 1964; Albrecht et al., 1996; Burela & Martin, 2007). During mating, the male habitually secrets mucus from the outer gland of the penis sheath, and the female feeds on it, a phenomenon that was interpreted as a nuptial feeding (Burela & Martín, 2007). At least under laboratory conditions, both males and females show a multiple mating behavior (Albrecht et al., 1996). Several authors observed the mating behavior in other ampullarids (Guimarães, 1981; Bahl, 1928; Nono & Mane, 1931; Demian & Ibrahim, 1971; Berthold, 1989, 1991). All of the descriptions agree on the basic pattern. Matings are subaquatic, and the male's foot adheres to the right side of the last whorl, while gripping the rim of the female's shell with the penis sheath.

Beyond the practical aspect of saving time of observation in experimental reproductive studies (Trigwell et al., 1997), the recognition of a snail's intention to mate or, contrarily, to avoid it, is an important aspect in the study of sexual preferences and mate choice. The aim of this study is to describe the sequence of actions or states that can eventually lead, but not necessarily does, to ova fertilization, and to quantify the frequency of the alternative pathways in the mating behavior of *Pomacea canaliculata*.

MATERIAL & METHODS

Both adult snails and egg masses were collected in the Curamalal stream at Pasman town, Buenos Aires Province, Argentina (37°14'31"S, 62°08'04"W), from a population that has been the subject of previous studies (Martín & Estebenet, 2002; Estebenet & Martín, 2003). Egg masses were incubated at room temperature until hatching. Hatchlings and field snails were kept in aquaria of appropriate size in a rearing room at 25 ± 2°C, under 14 h light per day and fed with lettuce *ad libitum*; water was changed and aquaria cleaned once a week. Field snails were sexed by the shape of the shell aperture and operculum (Estebenet et al., 2006); hatchlings were examined weekly to sex them as soon as possible, carefully seeking evidence of the testicle through the translucent shell. Females and males were reared separately.

Observation trials were performed in 3 liter glass aquaria, with water at 24–26°C and permanent illumination, in order to observe the mating behavior of isolated couples. One male and one female were put at the same time in the aquarium, without food, usually at 8:00 A.M. In each trial, up to 15 aquaria were simultaneously set up and the snails in them were observed continuously for 24 h.

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During the observation period each single state adopted or action performed by one or both mates (e.g. female passive, male crawling over the female's shell, mate probing) was recorded and chronometered. The frequency (%) of transition between two successive actions or states was estimated on the basis of the number of snails or mates that performed or adopted the first action or state; hence the sum of the frequencies of the alternative paths after an action or state is always 100%. When an action was repeated, the number of repetitions (mean ± SD) was recorded.

In order to schematize the mating behavior of *P. canaliculata,* the set of states and actions was divided in four phases (adapted from Trigwell et al., 1997) to account for apple snail particularities): precourtship, courtship, copula and poscopula. The precourtship was operatively defined in our study as the period from the moment when snails were paired in the aquarium to their first encounter and contact. Courtship begins when the male mounts the female shell and ends when the male adopts the copula position in order to insert its penis sheath. The copula starts when the male inserts the penis sheath into the female's pallial cavity and ends when it withdraws it. The postcopula phase was defined as the period in which the male remains on the female without genital contact. The duration (mean ± SD) of each phase was estimated on the basis of the snails or mates that completed that phase.

Observations of precourtship, courtship and the first part of copula phase (up to the moment in which the female enters a passive state after sheath intromission; Fig. 1) were performed with virgin snails reared in the laboratory. Males (n: 50, 30–35 mm of shell length, SL) and females (n: 50, 35–40 mm SL) were randomly paired; if they did not mate after 2 h of observation, they were reused after at least 24 h. A total of four hundred pairs were arranged during the trials; the pairs that ultimately engaged in copula were separated after pre-fixed times as part of a study on sperm transference, which will be published elsewhere.

Observations of the last part of copula and postcopula were performed with adult snails that presumably had mating experience: field females (n: 15, 40–50 mm SL) and males reared in the laboratory (n: 40, 31–43 mm SL) that had previously had contact with females (i.e. shared the same aquarium). Males and females were randomly paired and reused after at least 24 h; a total of 32 couples commenced sperm transference after the females had entered the passive state. All the behaviors registered during the first set of observations (virgin snails) were also observed in the second one (experienced snails), but only these 32 couples were used in the calculations of transition frequencies in the last part of copula and poscopula (Fig. 1).

RESULTS

Of a total of 400 pairs formed in the observation aquaria for the precopulatory behavior, only 191 (47.75%) engaged in some kind of interaction, initiating the precourtship phase (Fig. 1). Mate encounter comprises all the movements of the snails in the aquarium up to the moment of the first physical contact; in most cases, it was the male that approached the female and this occurred both when the later was moving or laying motionless. In one half of the mate encounters, the male mounted the female shell directly. In the other half, mate probing was observed: contacts with cephalic tentacles, labial palps or feet, radular scraping on shells or snouts, etc. This probing was generally performed by the males. The mean duration of precourtship was very variable: from a few seconds (if no probing occurred) to 2.29 h.

Only 147 out of the 191 pairs (76.96%) reached the courtship phase, with the male mounting the female shell (i.e., the male's foot completely lost its hold on the aquarium); in many cases, the males crawled counterclockwise over the female shell (viewed from the female apex; Fig. 2A), but this circling behavior was not quantified. After this, the male frequently dismounted spontaneously from the female's shell. Otherwise, the male began the positioning in order to gain foothold on the right (sutural) side of the female's last whorl and to near the sutural side of its aperture to that of the female. The male's positioning commonly elicited a swinging reaction by the female (Fig. 2B): the female rotated its shell several times in a counter- and clockwise way, with the foot adhered to the substratum. After a short pause, this swinging reaction could be repeated up to four times by the female. The female shook off the mounting male in almost 60% of the cases. If not, after the swinging the female entered a passive state (motionless and firmly adhered to the aquarium) with the male still mounted.

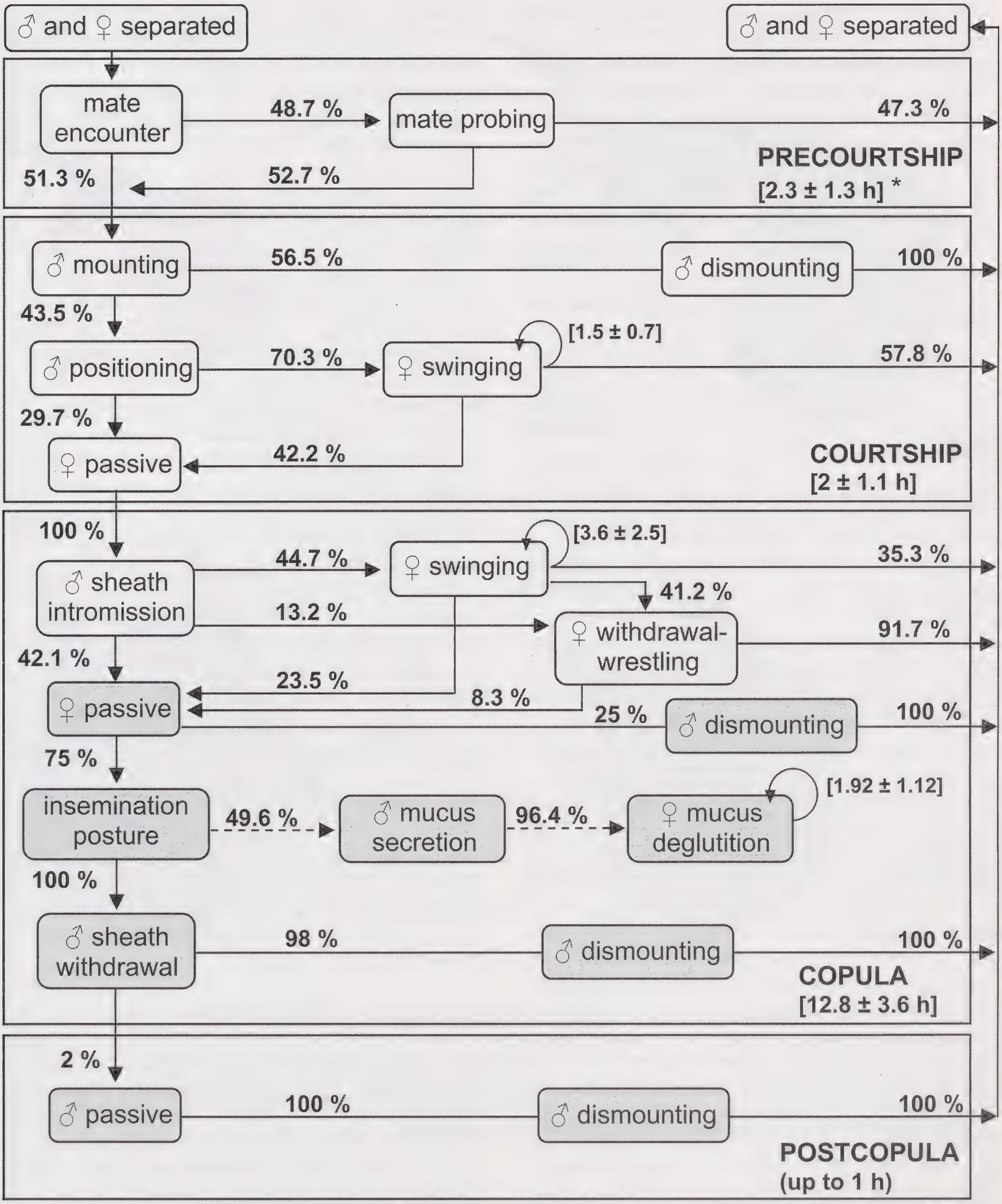

FIG. 1. Descriptive scheme and statistic summary of mating behavior in *Pomacea canaliculata*. Rounded boxes represent single states or actions performed by one or both mates. Percentages are the frequencies of the different pathways that snails followed after each action or state. Solid arrows connect successive actions or states; hatched arrows join two actions that can occur simultaneously and curved arrows indicate repeated actions (number of repetitions [mean ± SD]). Straight boxes include the different actions or states of each phase; phase durations [mean ± SD] are shown (* calculated only for the cases where mate probing occurred). White boxes and the arrows arising from them correspond to virgin snails whereas gray boxes and their arrows correspond to snails with mating experience.

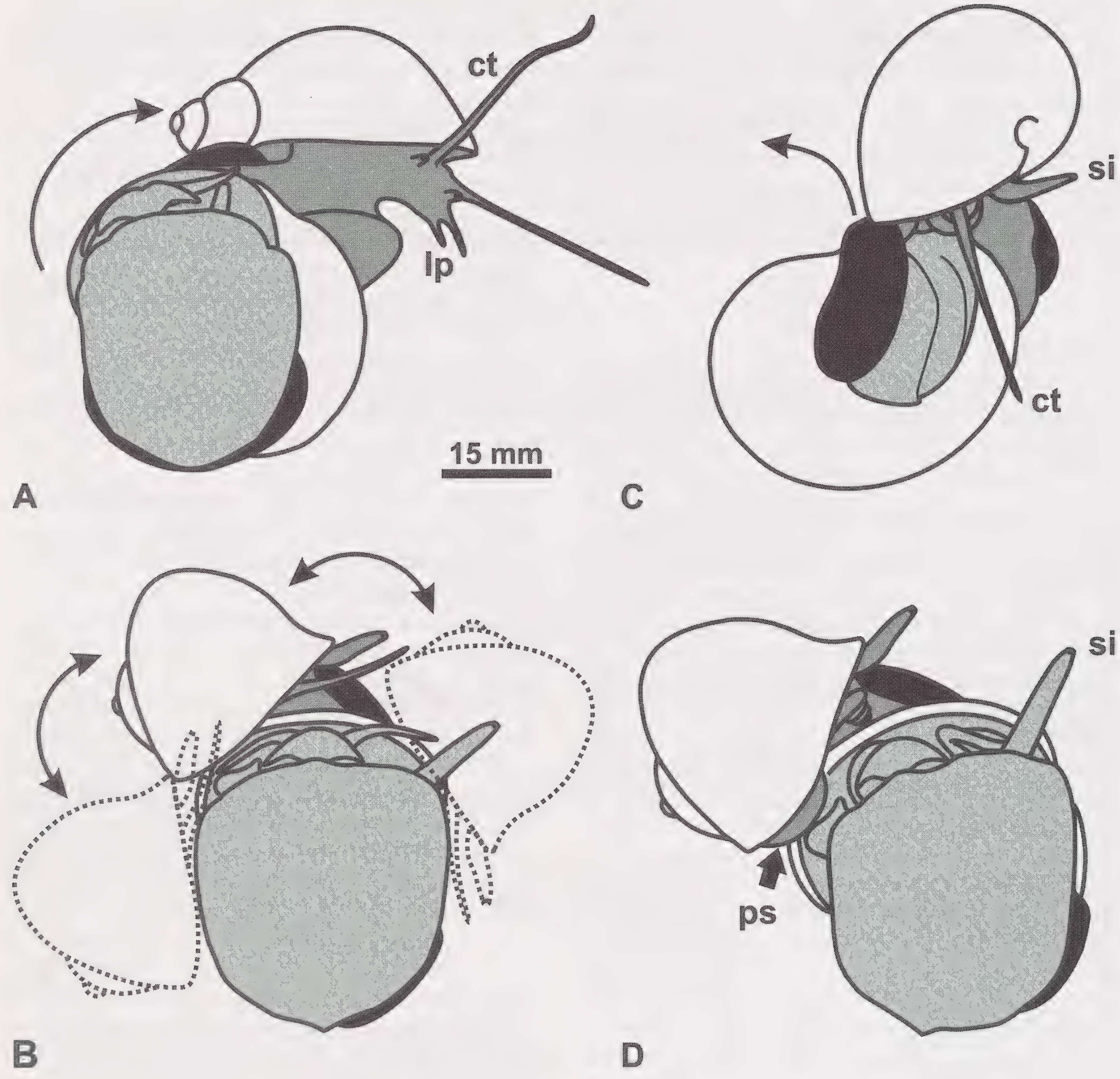

FIG. 2. Schemes of actions or states that occur during the mating behavior in *Pomacea canaliculata* (drawn on the basis of photographs taken in aquaria; water surface is located towards the upper side in all cases and the female's foot is adhered to the aquarium wall, except in C). A: Circling; B: Swinging; C: Withdrawal-wrestling; D: Insemination posture. Female body (light gray), male body (dark gray), shells (white) and opercula (black), si (siphon), ct (cephalic tentacle), lp (labial palps) and ps (penis sheath); curved arrows indicate the direction and sense of movement.

The duration of the courtship was quite variable, ranging from 0.32 to 4.77 h; on the whole, only 38 pairs (19.89%) completed the courtship phase and began copula.

The intromission of the penis sheath initiates the copula phase; this action elicited strong reactions in 57.90% of females. Most of them performed several swinging events that shook off the male in a third of the cases. A 13.16% of the females that suffered the intromission of the sheath reacted immediately loosening its foothold on the aquarium, falling to the bottom, withdrawing the cephalopodium inside the shell, and trying to close the aperture with the operculum; if the male did not retrieve the sheath, the female engaged in a wrestling behavior, trying to push the sheath out of the pallial cavity with the operculum (Fig. 2C). A 41.18% of the females that showed swinging also performed the withdrawal-wrestling behavior afterward. Almost all of the females that performed this behavior interrupted the copula. On the whole, in 73.96% of the cases, the female finally entered a passive state, tightly adhered to the aquarium, after the sheath intromission. In a few cases, after 20–60 minutes, the male spontaneously retrieved the sheath and then dismounted the female shell, without any apparent cause.

In cases when spontaneous quitting did not occur, the male also entered a passive state similar to the female's while tightly adhered to its shell. Both members of the couple usually then remained in this position with their cephalic tentacles coiled (insemination posture, Fig. 2D), and apparently sperm transference began. A direct observation of this process (including the contact of the tip of the penis with the female gonopore) was not possible although it certainly occurred, since laying of fertilized eggs commenced a few days later. During sperm transference, the female was usually motionless but occasionally moved around to ventilate the lung; sometimes the male took advantage of this last behavior to do the same. After a minimum of four hours, in many cases it was possible to observe that the outer sheath gland began to secrete a whitish mucus that accumulated in the form of a small drop on the gland opening; in most cases, the mucus drop was degluted at irregular intervals by the female. After 12.80 hours on average (ranging from 4.13 to 19.78 h), the male slowly retrieved the sheath, dismounted the female shell and the copula ended. Only in a few cases, the male remained on the female's shell for up to one hour with the sheath folded in resting position inside its own pallial cavity (postcopula phase).

DISCUSSION

The observations on mating behavior of *Pomacea canaliculata* reported on here are in agreement with those of previous non-systematic studies (Andrews 1964; Berthold, 1989, 1991; Schnorbach, 1995; Albrecht et al., 1996); however, a number of new behaviors, especially those that can be interpreted as mate-rejection actions by the female, are reported for the first time.

Almost half of the virgin snails paired in the trials did not show any type of interaction even when they shared an aquarium for two hours. Probably some of these virgin snails, specially the females, were still immature, despite the fact that they were larger (35–40 mm SL) than the lower limit reported previously to begin egg laying (25 mm SL; Estebenet & Martín, 2002; Tanaka et al., 1999). However, it has been recently reported that females reach maturity at different shell lengths and ages, depending on food availability: females taking 32 weeks to mature (the age of virgin females used in the present study) began to copulate at 40 mm of shell length (Tamburi & Martín, 2009).

The precourtship phase, especially the active mate search, was unnatural in our study, because mates were paired randomly in a small aquarium. Takeichi et al. (2007) provided evidence that in the laboratory *P. canaliculata* snails are able to detect conspecific's waterborne odors and also to follow their mucus trails. Darby et al. (1997) suggested that *P. paludosa* males can follow females' trails into funnel traps in a restored marsh in Florida. However, the existence of specific mate recognition mechanisms in *P. canaliculata* is unclear, because Albrecht et al. (1996) recorded males in copula with other males when maintained together without females for two weeks. In all-male rearing tanks, routinely maintained in our laboratory, such homosexual couples were frequently observed, either for virgin or for experienced males, but never in tanks where males and females were reared together.

At the beginning of the courtship phase many males performed a counterclockwise circling behavior over the female shell. Such behavior has been described for freshwater pulmonates and interpreted as an assessment of partner size (De Boer et al., 1996; Koene et al., 2007). Perhaps this behavior has the same meaning in *P. canaliculata*, because males prefer to mate with big females, at least under laboratory conditions (Estebenet & Martín, 2002).

Swinging behavior was elicited in the courtship phase when the male adopted the copula position on the female's shell but not when it mounted the shell; swinging was rarely observed in rearing tanks when a snail was mounted by other. Two possible explanations can be stated for this behavior: the female could perform the rotations to evaluate male mate quality (weight, vigor or motivation) or to reject a male that showed a clear intention to begin copula. At least an explanation based on male size seems unlikely because it is neither important in access to females (Estebenet & Martín, 2002; Estoy et al., 2002a) nor in female reproductive output (Tamburi & Martín, 2009). Mate rejection is the most commonly accepted explanation for swinging behavior in freshwater pulmonates (e.g., DeWitt, 1991; Trigwell et al., 1997; Facon et al., 2006); swinging is also used as an avoidance response to predatory leeches in this group (e.g., Townsend & McCarthy, 1980; Wilken & Appleton, 1991) and also in small juveniles of *Pomacea bridgesii* (less than 13 mm; Aditya & Raut, 2005).

Swinging or withdrawal-wrestling behaviors, sometimes combined, were triggered when the male inserted the penis sheath in the pallial cavity of the female. Withdrawal-wrestling behavior can be considered a more definitive mate rejection, since it forced the male to quit in almost all cases. Nono & Mane (1931) described a similar female response to male mounting in the Philippine apple snail *Pila luzonica* and attributed it to a lack of sexual receptivity. Operculum closing is a defensive response to predatory leeches in some species of small freshwater prosobranchs (Kelly & Cory, 1987) and in juveniles of *P. bridgesii* (Aditya & Raut, 2005), but probably not in adults of *P. canaliculata* since they can harbor entire populations of leeches and temnocephalids in their pallial cavities (Vega et al., 2006). Operculum closing seems not to be merely an alarm response to the entering of a strange body inside the pallial cavity. When considered together with the expulsion of the sheath from the pallial cavity, it appears as a clear mate rejection response.

One feasible reason for a female to avoid copula is that it is physiologically ready to deposit an egg mass. This explanation does not apply in the present case, because all females were virgin, and virgin females do not lay eggs (Gamarra-Luques et al., 2006). Females of this species are able to store sperm and to lay viable eggs for up to 140 days and also after winter resting (Estebenet & Martín, 2002), so that the benefits of multiple matings for females are not clear. Forced matings have been reported in other invertebrate taxa (e.g., Cordero, 1999) but they would apparently be rare in *P. canaliculata*, considering the effectiveness of the withdrawal-wrestling behavior. Estoy et al. (2002a) reported that virgin females with immature albumen glands, and therefore not able to lay eggs, nevertheless copulate with mature males in the laboratory. Perhaps these immature females rejected the first mating attempts but eventually accepted the mates (i.e., entered in a passive state) due to the fact that they remained paired in the same aquarium during the entire experiment. Facon et al. (2006) noted that mate rejection behaviors are probably more effective in the field than in no-choice experiments where both partners are artificially maintained isolated together for extended periods.

In some cases, the male quitted spontaneously after the female entered the passive state. It could be that the male failed to contact the female gonopore with the T-shaped sulcus of the penis sheath and to adhere to the pallial cavity (Gamarra-Luques et al., 2006) or perhaps the last rejection resource of the females is to close the gonopore.

Presumably after the contact of the penis sheath with the female gonopore, the penis is introduced in the feminine duct. According to Bahl (1928), only the penis tip penetrates in the Indian apple snail *Pila globosa* and the presence of a heavily ciliated sperm groove in *P. canaliculata* (Catalán et al., 2002) suggests that the penis does not go too far in this case either. The sperm transference begins at least one hour after the intromission of the penis sheath (unpub. results), and no further negative reaction was observed thereafter. During this phase, the male's outer sheath gland releases a mucus secretion that is eaten by the female, a behavior that has been interpreted as a nuptial feeding to entice the female to remain in copula (Burela & Martín, 2007). After several hours and without any externally observable reason, the males withdraw the penis sheath and dismount; whether the male quits due to sperm depletion or because the female closes it gonopore to interrupt the transference cannot be determined for now.

Yusa (2004, 2007) reported that double paternity occurs in a single egg mass of *P. canaliculata*, suggesting that sperm mixing occurs within the female seminal receptacle and that sperm competition could be an important sexual selection mechanism in this snail. The generally brief and infrequent postcopula phase indicates that, if males display a mate guarding behavior to prevent a subsequent mating, it would be during the copula phase (*in copula* guarding *sensu* Sillén-Tullberg, 1981) or triggered only by the presence of prowler males.

On the basis of differential copula frequencies in experiments with multiple partners of both sexes and two sizes, Estebenet & Martín (2002) concluded that *P. canaliculata* shows size-assortative mating. However, the experimental design did not allow discerning the size preferences of each sex. The mate rejection behaviors displayed by females in the present study are interpreted as evidence that females are able to perceive a male's intention to mate. The knowledge of these behaviors will allow the study of sexual preferences (Jennions & Petrie, 1997), a necessary step to understand mate choice patterns in this snail.

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DESCRIPTION OF GLOCHIDIA OF THREE SPECIES OF FRESHWATER MUSSELS (UNIONIDAE) FROM SOUTHEASTERN TURKEY

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INTRODUCTION

Unionoidean larvae have a short parasitic phase on fish scales, fins, or gills (Mansur & Silva, 1999). Within in the family Unionidae, glochidia are classified as subcircular unhooked, subtriangular hooked with spinules, subtriangular hooked without spinules, or axe-headed (Graf & Cumming, 2006).

The glochidial stage of *Unio terminalis* Bourguignat, 1852, from the northeast Mediterranean region, has not been described previously. This species is an important component of the macrobiota of the inland waters of the region and the most abundant unionid in Gölbaşı Lake, Hatay Province, Turkey (Şereflişan, 2003). *Potomida littoralis* (Cuvier, 1798) is the second most abundant species, and *Anodonta pseudodopsis* Locard, 1883, is the least abundant species in this lake. These species can be diagnosed at the juvenile stage, but the glochidia of *Unio terminalis* and *Anodonta pseudodopsis* are difficult to distinguish.

The glochidial morphology and measurements based on light microscopy were described by Engel & Wächtler (1989) and other researchers. Using scanning electron microscopy (SEM), glochidial shells of family Unionidae were studied by Hoggarth (1999) and other researchers. Taxonomic and geographical diversity of the Unionidae were examined by Graf & Cumming (2006). Certain reproductive characteristics of *Unio terminalis* were reported by Çek & Şereflişan (2006), and the periods of gravidity were reported by Şereflişan (2003).

With the use of scanning electron microscopy, minute glochidial structures can be examined and used to interpret relationships among unionid species. This paper describes the glochidia of the northeast Mediterranean mussels *P. littoralis, A. pseudodopsis*, and *U. terminalis*.

MATERIALS AND METHODS

Gravid female *P. littoralis*, *A. pseudodopsis,* and *U. terminalis* were dredged from 0.5–2 m in Gölbaşı Lake, Hatay, Turkey (36°30'16"N; 36°29'42"E) from September 2003 to June 2004. Some specimens were also retrieved from 5–6 m by SCUBA diving. Samples were transported live to the laboratory in cool boxes and kept in aquarium conditions for 1 week.

Mature glochidial larvae were released by squeezing the marsupial gills in charcoal-filtered tap water. The larvae were washed several times with water. Whole glochidia were placed open on a slide and examined and photographed both fresh and after being fixed and stained.

In order to stain the preparations samples of each species were first fixed in glutaraldehyde in 50 mM phosphate buffer (pH 7.2) and transferred into 50% ethanol. The samples were then dehydrated in an ethanol series of 60%, 70%, 80% for 30 min in each. The samples were mounted on stubs with conductive double-sided carbon tape and coated with gold/palladium in a sputter coater (Polaron SC7620, UK) for 90 sec at 9 mA. The samples were examined and photographed using a JEOL JSM 5500 scanning electron microscope (SEM) at an accelerating voltage of 5 kV.

Glochidial valve length was measured as the greatest distance from anterior to posterior margins parallel to the hinge. Glochidial valve height was measured as the greatest distance from dorsal to ventral margins perpendicular to the length.

RESULTS

Potomida littoralis (Cuvier, 1798)
Figs. 3, 5A, 6–11

The glochidia of *P. littoralis*, which are parasitic on the gills of freshwater fish, have no hooks. They are subelliptical, with a semicircu-

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TABLE 1. Some characteristics of unionacean glochidia.

	Shell colour	Shell form	Hooks	End of hook
Potomida littoralis	Lightly brownish	remarkably longer than high	no hooks with spines	
Anodonta pseudodopsis	cream-brown; sometimes cream coloured	slightly longer than high	medium-size (72–76), with numerous spines	pinched, triangular sharp
Unio terminalis	cream-brown usually whitish brown	slightly longer than high with prominent ventral apex	medium-size (31–33), with numerous spines	triangular sharp, pinched

lar ventral margin. The lateral margins are subequal, with the anterior margin slightly thicker than the posterior. Lanceolate micropoints are located on the ventral rim of valve and on the narrow ventral flange, arranged in broken vertical rows covering most of the area of flange. These glochidia can be distinguished from other species in being more broadly rounded, with numerous micropoints.

Anodonta pseudodopsis Locard, 1883

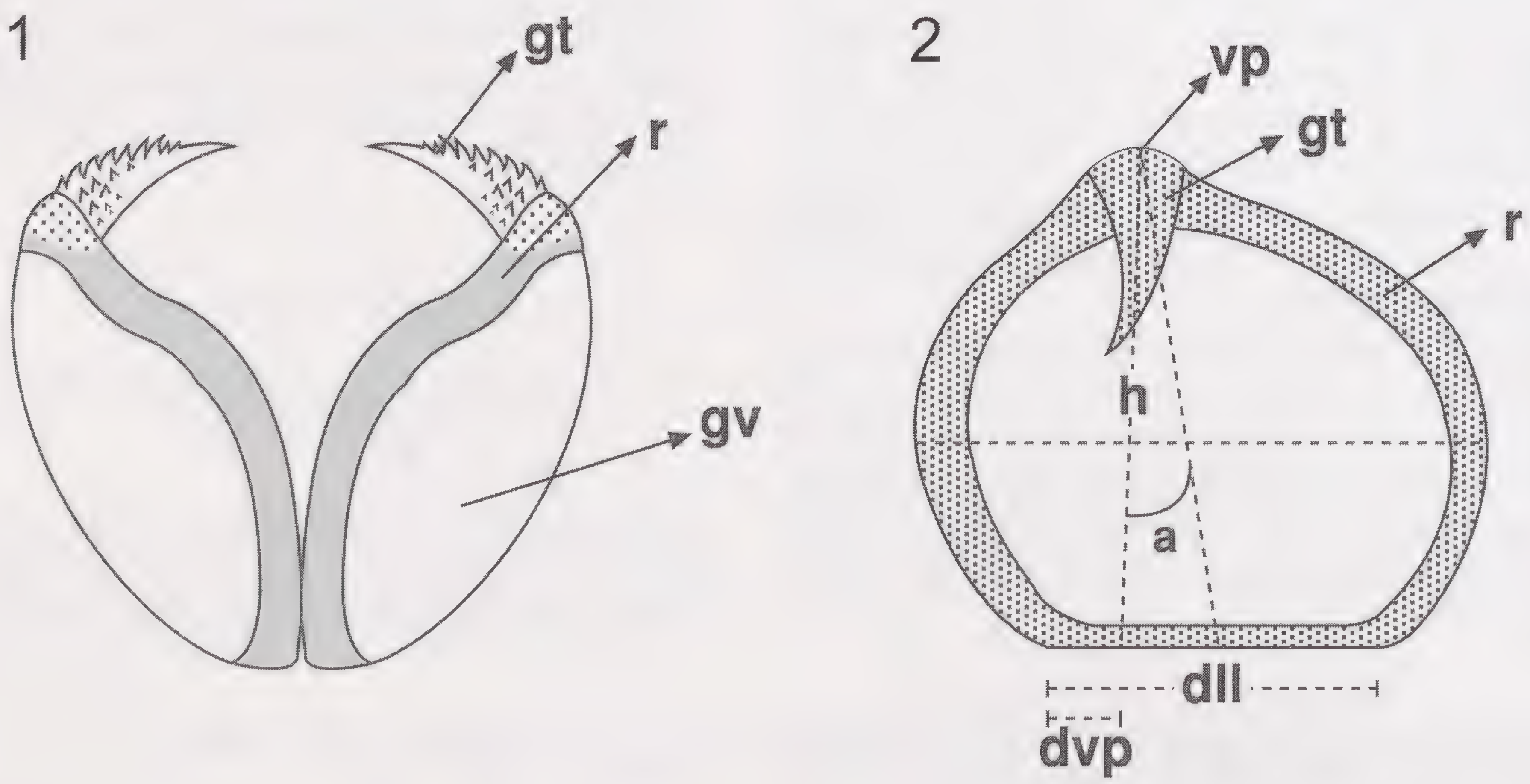
Figs. 1, 2, 5B, 12–17

The glochidia of *A. pseudodopsis* are fin parasites. They are subtriangular and have a rounded anterior margin, which is smoother than the posterior. The posterior margin is gently curved throughout its length, producing a moderately asymmetric contour. Styli form a hook with biconcave lateral margins and a sharp tip. Hook styli form is covered with about 12 microstylets. Numerous micropoints cover the ventral terminus and lateral surface of the hook. The unsculptured distal tip has a tapered margin. The valves of the glochidia of *A. pseudodopsis* are usually a light coloured cream-brown.

Unio terminalis Bourguignat, 1852

Figs. 5C, 18–25

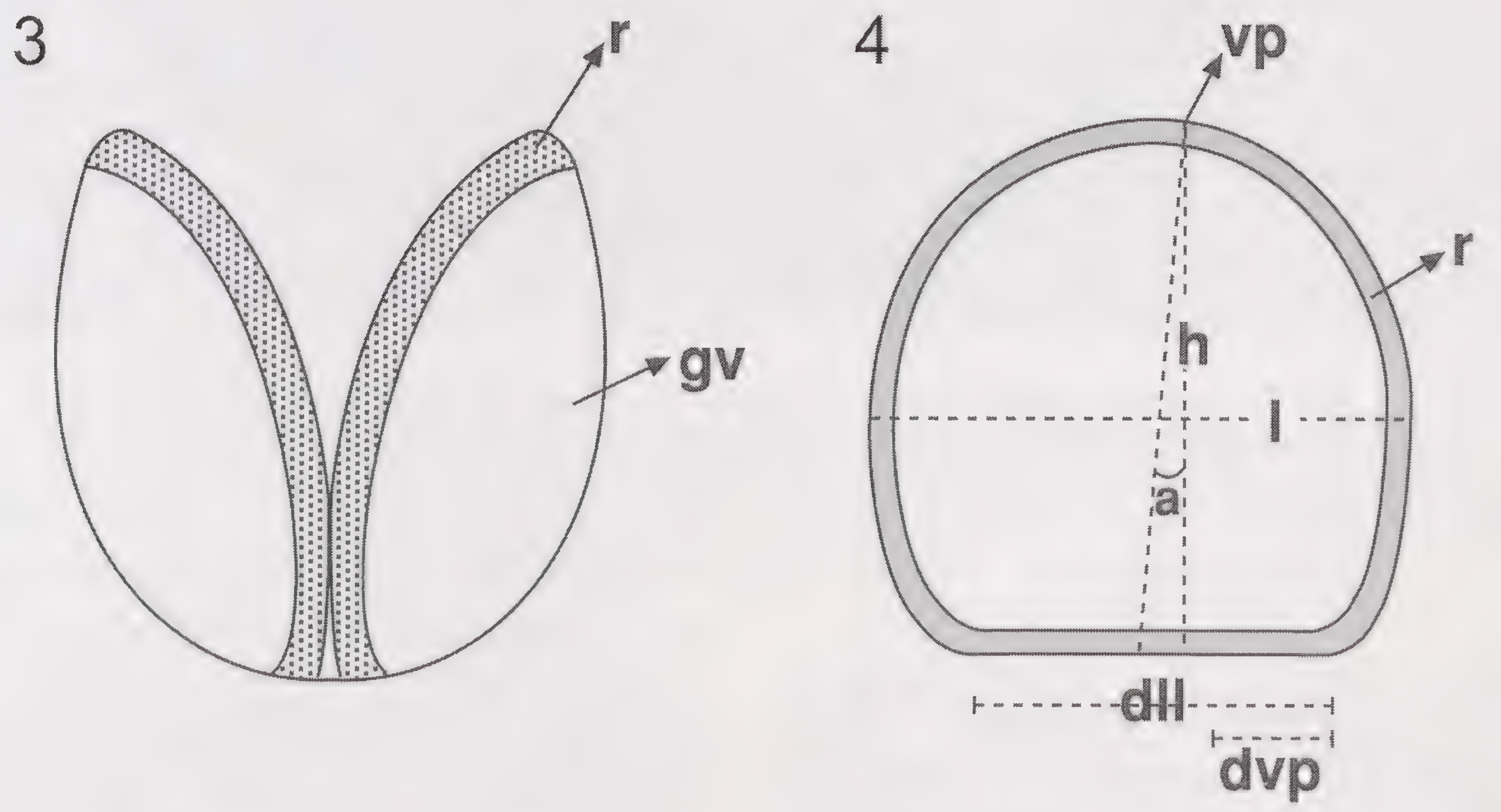
The glochidia of *U. terminalis* are fin parasites. They are subtriangular with a slightly asymmetric shape. A styliform hook extends from ventral terminus of each valve. The hooks are uniformly tapered. Most microstylets (about 30) are bluntly pointed, whereas some are more proximally multifaceted. Micropoints cover the lateral and proximal margins of the hook, except for a narrow distal band. The cream-brown valves of the glochidia are translucent.



FIGS. 1, 2. Glochidium of *Anodonta pseudodopsis*, with a hook-shaped teeth. FIG. 1: Frontal view, profile of teeth; FIG. 2: Glochidial valves in lateral view.

TABLE 2. Statistical data on glochidia. Abbreviation: DLL, Dorsal hinge length; DVP, Displacement of the ventral point in relation to the middle of the hinge lenght; A°, Angle of the ventral point in relation to the center of the hinge; GTL, Glochidial tooth length; GTH, Glochidial tooth height.

	Range	Mode	Mean	s	CV%
Potomida littoralis (Lamarck, 1801) N= 60					
Length	190–280	240	235	0.0081	2.97
Height	132–174	150	153	0.0093	6.72
DLL	122–146	130	134	0.0095	3.21
DVP	35–61	50	48	0.0072	20.26
A°	3°–10°	7°	6°	1.76	14.42
Anodonta pseudodopsis (Locard, 1883) N= 60					
Length	310–346	325	328	0.0112	2.46
Height	292–314	300	303	0.0077	2.37
DLL	230–291	250	260.5	0.0119	2.65
DVP	20–50	35	35	0.0085	21.31
A°	7°–17°	12°	12°	1.93	16.42
GTL	72–76	74	73	0.0092	3.69
GTH	21–27	24	24	0.0078	4.35
Unio terminalis (Lea, 1863) N= 60					
Length	193–247	220	220	0.0109	3.41
Height	175–196	180	185	0.0085	2.97
DLL	170–213	190	191	0.0105	4.32
DVP	35–68	50	51	0.0088	22.54
A°	6°–15°	11°	10°	1.89	16.93
GTL	31–33	32	32	0.0071	2.51
GTH	10–12	11	11	0.0055	2.13



FIGS. 3, 4. Glochidium of Potomida littoralis, unhooked glochidium type. FIG. 3: Frontal view; FIG. 4: In lateral view. Abbreviation: a – angle; am – adductor muscle; r – rim; l – length; dll – dorsal hinge; dvp – displacement of the ventral point in relation to the middle of the hinge; gb – growth band; gt – glochidial tooth; gv – glochidial valve; h – height; vp – ventral point.

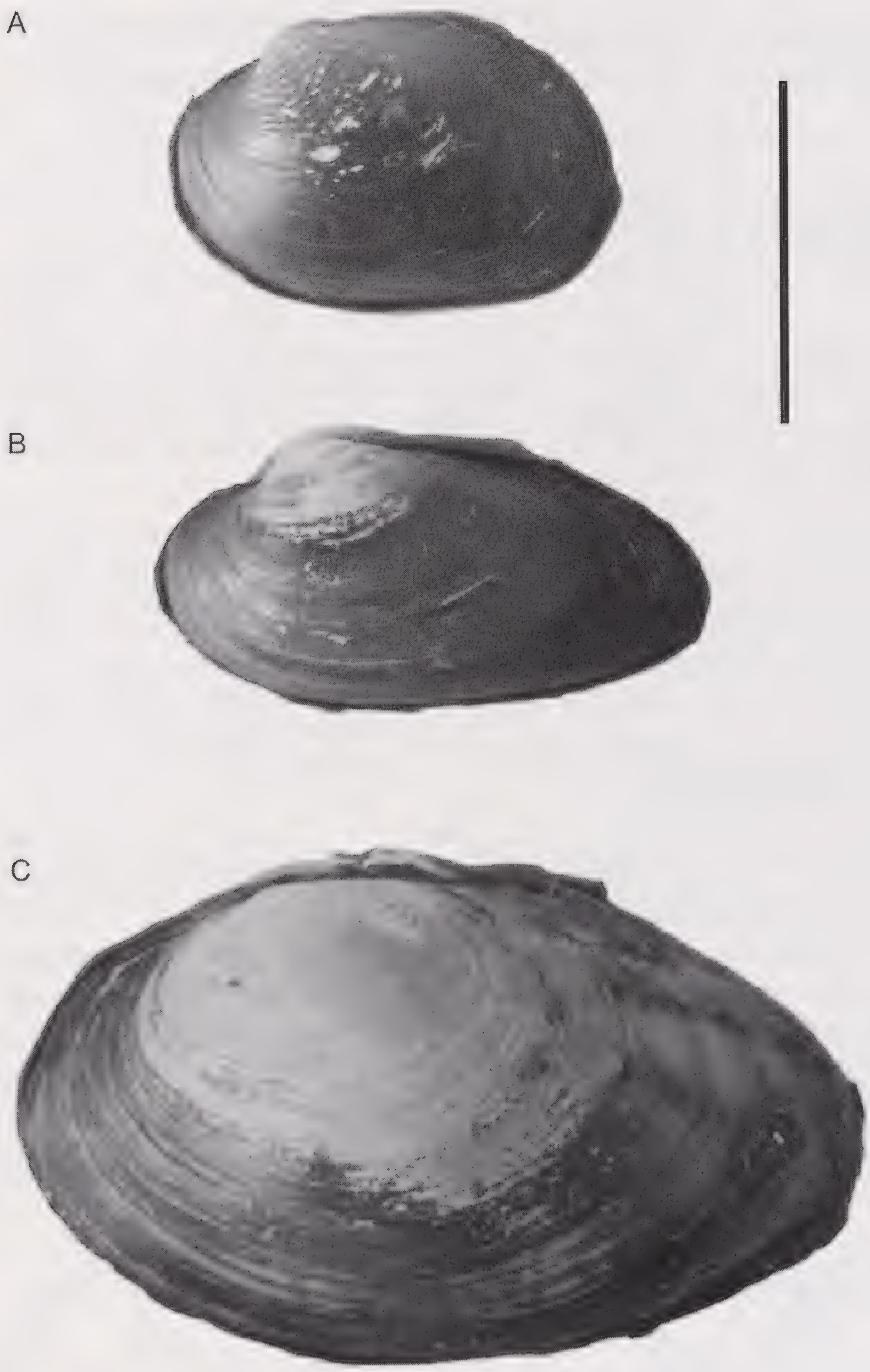


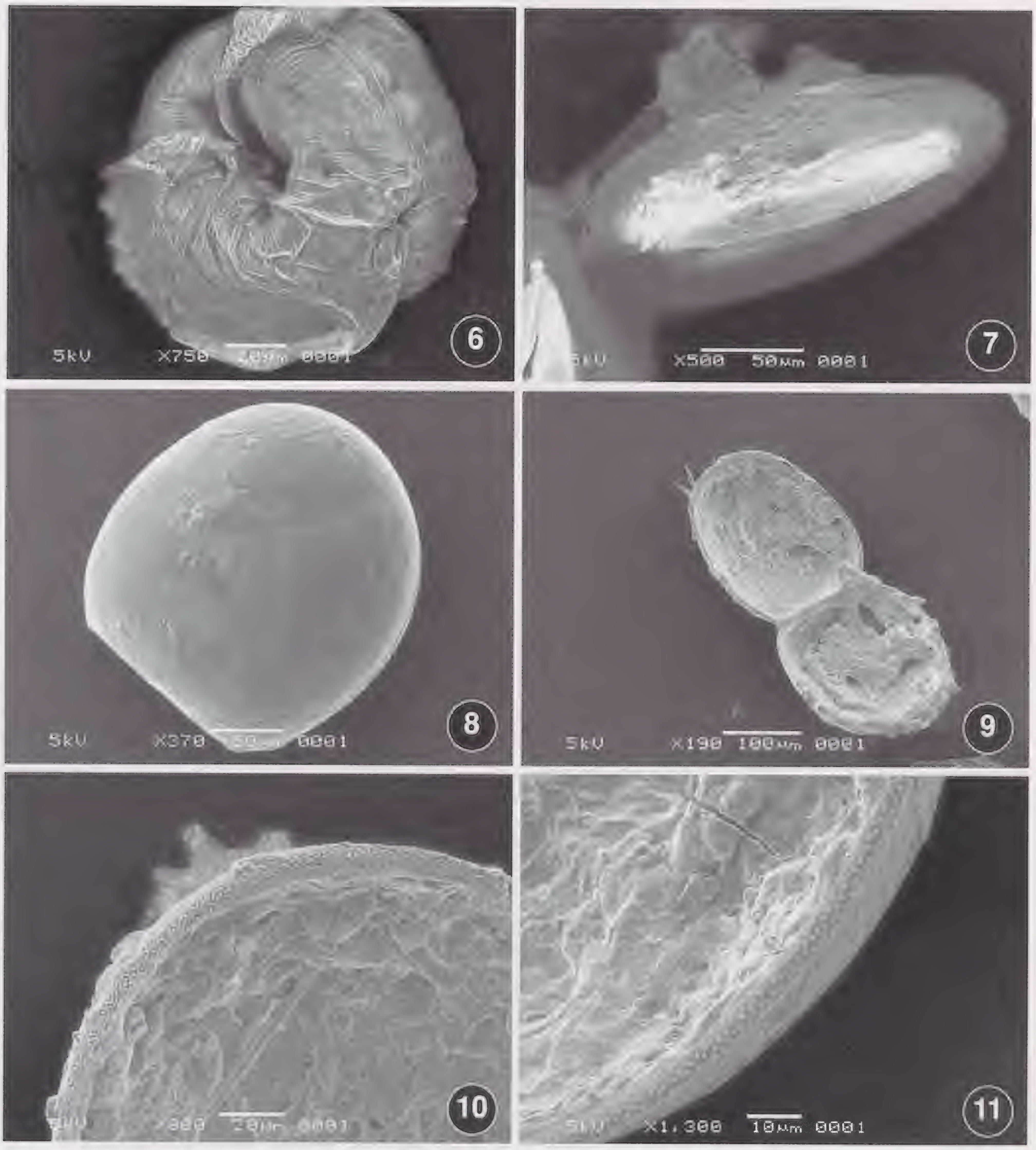
FIG. 5. Shells of the adult freshwater mussels. A: *Potomida littoralis*; B: *Unio terminalis*; C: *Anodonta pseudodopsis* (scale bar = 5 cm).

DISCUSSION

Freshwater mussels spend some time as larval stage parasites on freshwater fishes. Hooked glochidia may attach themselves to the body surface, gills, or fins. Hookless glochidia are usually encysted on fish gills (Arey, 1924; Wächtler et al., 2001). Unhooked-type glochidia are observed in the Unionoidae, including *Potomida* (Graf & Cummings, 2006).

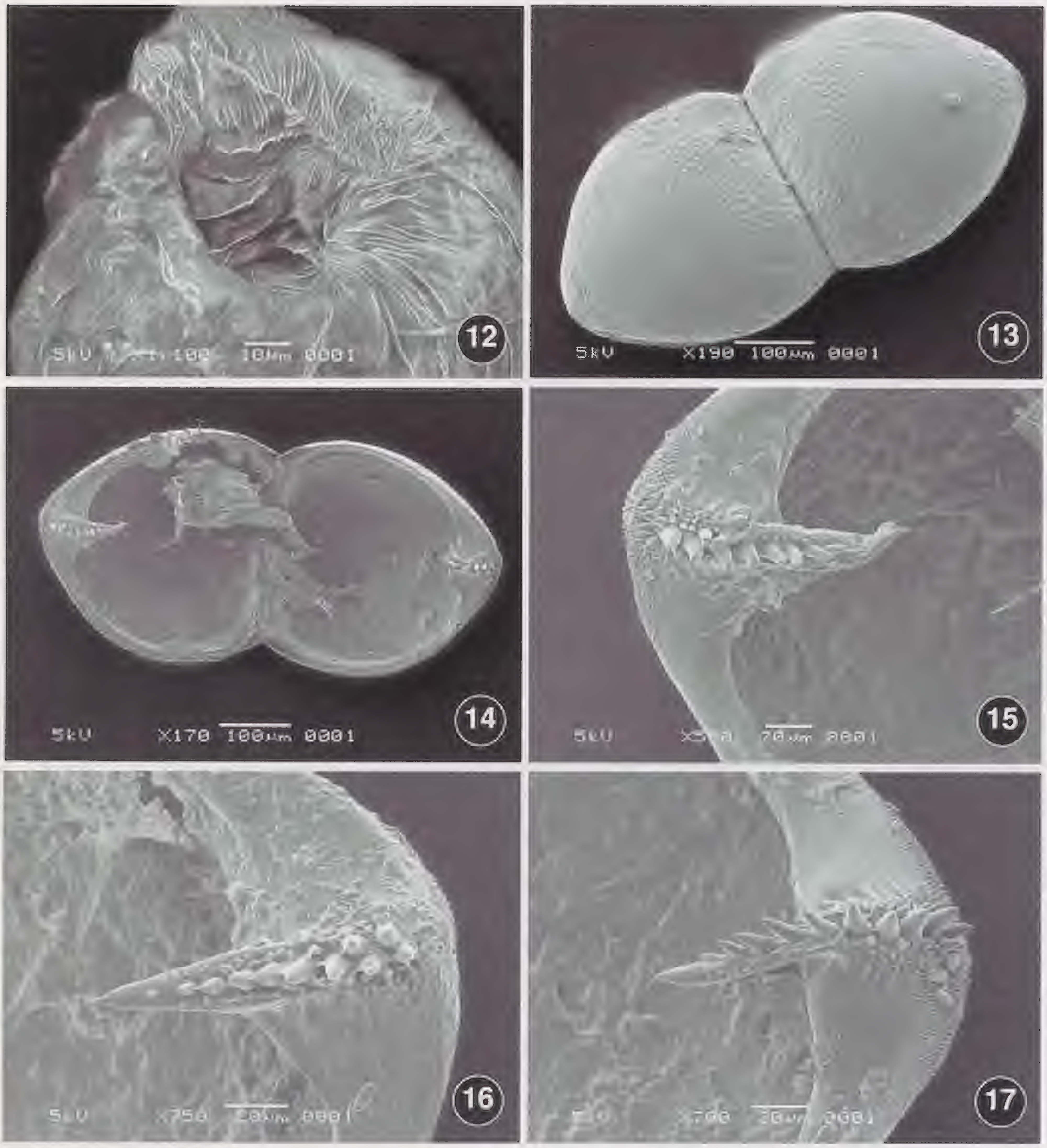
In this study, *P. littoralis* was identified by its subelliptical shape (length 190–280 µm, height 132–174 µm). It is very thin. In Europe, hookless but denticulate glochidia include at least those of *P. littoralis*, according to Giusti (1973).

The glochidia of *A. pseudodopsis* can be distinguished from those of *Unio* by their shape, hook structure, exterior valve sculpturing, and size. The dimensions of the larva of *A.*



FIGS. 6–11. Scanning electron microscopy of the glochidia of *Potomida littoralis* in the marsupium. FIG. 6: The shell pellicle is saddle shaped; FIG. 7: Differentiation of the shell from late gastrula to glochidia; FIG. 8: Surface views of *P. littoralis*; FIG. 9: Inside views of opened *P. littoralis* glochidia; FIGS. 10, 11: Numerous bluntly pointed micropoints present on valve margin.

pseudodopsis ranged from 310 µm to 346 µm in length. They resemble those of *A. cygnea* (Linnaeus, 1758) – 0.31–0.43 mm (Claes, 1987; Niemeyer, 1992) – in shape and size, but have no other characters of that species. The *A. pseudodopsis* glochidia different from those of *A. anatina* (Linnaeus, 1758) – 0.34–0.36 mm (Niemeyer, 1992), *A. beringiana* (Middendorff, 1851) – 0.28–0.29 (Hoggarth, 1999), *Pseudoanodonta complanata* (Rossmässler, 1835) – 0.29–0.34 mm (Hüby, 1988; Kinzelbach & Nagel, 1986), and other species of the Anodontinae in view of their numerous microstylets and micropoints. The glochidia of *A. pseudodopsis*

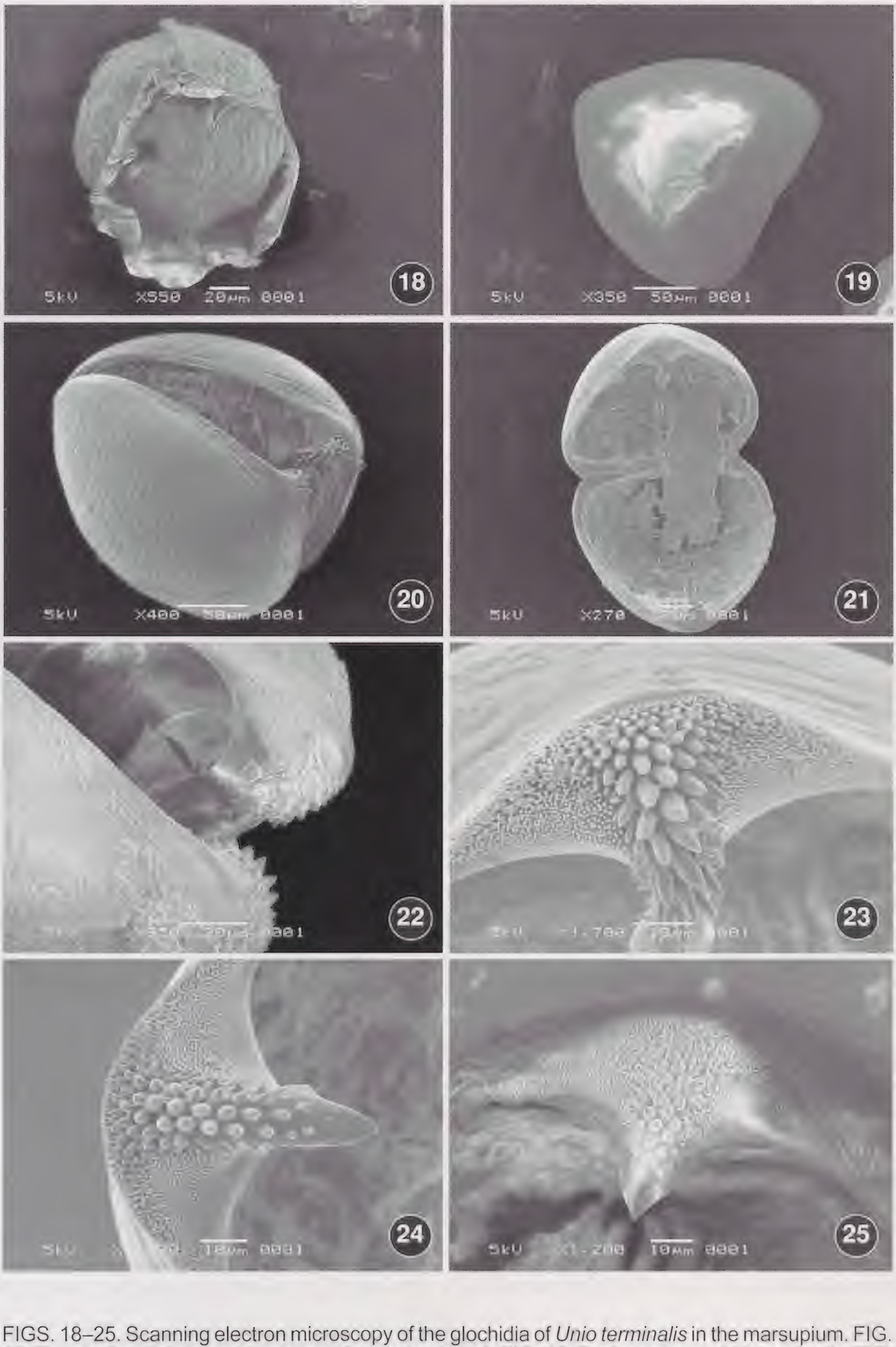


FIGS. 12–17. Scanning electron microscopy of the glochidia of *Anodonta pseudodopsis* in the marsupium. FIG. 12: The shell-pellicle is saddle shaped (gastrula phase); FIG. 13: Surface views of *A. pseudodopsis*; FIG. 14: Inside views of opened *A. pseudodopsis* glochidia; FIGS. 15–17: Styliform hook (about 11 microstylets) of *A. pseudodopsis* glochidia.

were not previously figured.

The hook structure of *U. terminalis* differs from those of *U. tumidus* Retzius, 1788, and *U. pictorum* (Gmelin, 1791) not only in being tapered but also in the placement of microstylets on the hook. The glochidia of *U. terminalis* are darker than those of A. *pseudodopsis* and have a very long, pointed hook with several stylets of various lengths arranged in multiple rows. No previously published figure of this glochidium was found.

Examination of glochidia of additional species would provide information relevant for unionid taxonomy.



FIGS. 18–25. Scanning electron microscopy of the glochidia of *Unio terminalis* in the marsupium. FIG. 18: The shell pellicle is saddle shaped (gastrula phase); FIG. 19: Surface views of *U. terminalis*; FIG. 20: Lateral view; FIG. 21: Inside views of opened *U. terminalis* glochidia; FIG. 22: Early glochidial stage; FIGS. 23–25: Syliform hook with about 30 microstylets of *U. terminalis* glochidia.

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COMPARATIVE CYTOGENETIC ANALYSIS OF THREE STYLOMMATOPHORAN SLUGS (MOLLUSCA, PULMONATA)

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INTRODUCTION

The Stylommatophora include 20,000 to 30,000 extant species of land snails and slugs, belonging to 70–90 families. Their classification system is still controversial because phylogeny and systematic relationships at the family level are poorly understood. Besides morphological studies, ribosomal RNA genes and the H3/H4 histone gene cluster (Ambruster et al., 2005; Wade et al., 2006) have also been used to resolve the relationships within this order. Recently, by comparison of primary sequence of mitochondrial and nuclear genes, Grande et al. (2004) resolved the Stylommatophora as the early split monophyletic sister group of all the other gastropod taxa.

Available data on the cytogenetics of Stylommatophora are extremely poor (reviewed by Patterson, 1969, and Thiriot-Quièvreux, 2003) and mostly concern the haploid (n) and/or diploid (2n) chromosome numbers and karyotype morphology. Vinogradov (2000) determined genome size (GS) and genome base composition in 15 pulmonate species, with five being stylommatophorans. In the last few years, only two thorough cytogenetic investigations have been carried out on *Milax nigricans*, *Cantareus aspersus*, and *C. mazzullii* (Vitturi et al., 2004, 2005), and their results proved to be useful to examine: (1) genomic organization at molecular level using fluorescent *in situ* hybridization (FISH) with repetitive DNA sequences as probes; (2) reciprocal position of ribosomal (18S–28S and 5S rDNA) and telomeric $(TTAGGG)_n$ multigene families using simultaneous double-colour FISH; and 3) evolution of genome by comparison of genome size (C-value) and base-pair composition.

In the present paper, we describe a cytogenetic comparative analysis of *Limacus flavus* (Linnaeus, 1758) (Limacidae), *Tandonia sowerbyi* (Férussac, 1823) (Milacidae), and *Deroceras panormitanum* (Lessona & Pollonera, 1882) (Agriolimacidae), carried out by standard (i.e., Giemsa and fluorochrome staining), and molecular (*in situ* hybridization) methods. Percentage of adenine-thymine DNA (AT-DNA) and genome size (GS) were also investigated for the three species and, in addition, for *Milax nigricans* (Philippi, 1836) (Milacidae), still unexplored on these parameters. This study was carried out with the aim of contributing to the karyological knowledge of the order Stylommatophora, hitherto broadly neglected from this point of view. In addition, we wanted to test whether cytogenetic analysis could reveal any inter-specific genomic differences that may be considered as informative characters in the phylogenetic tree construction.

MATERIAL AND METHODS

The specimens of *Limacus flavus* (Linnaeus, 1758) (N = 80), *Tandonia sowerbyi* (Férussac, 1823) (N = 50), *Deroceras panormitanum* (Lessona & Pollonera, 1882) (N = 55), and *Milax nigricans* (Philippi, 1836) (N = 30) were collected in the lawns around Polizzi Generosa, northwestern Sicily, Palermo Province, during 2004, 2005 and 2006. Sexually mature individuals occurred only on September–October (*L. flavus*, *T. sowerbyi*, and *M. nigricans*) or October–November (*D. panormitanum*) of each year. Specimens were identified according to the guidelines of Giusti (1973). Systematics follows the scheme proposed by Bodon et al. (1995). Vouchers of all investigated species were deposited in the laboratory of cytogenetics and molecular genetics (University of Urbino, Via Maggetti 22).

Chromosomes were obtained by air-drying from sexually mature testicular lobes after *in vivo* colchicine treatment (0.5 mg/ml for 1 h at room temperature), observed with a Leica microscope, and photographed with a Kodak Ektacolor 800 ASA film. Chromosome classifi-

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cation is according to Levan et al. (1964) and the centromeric index was evaluated using the method proposed by Naranjo et al. (1983). Metacentric, submetacentric and subtelocentric chromosomes (m, sm, st) were considered as bi-armed while acrocentrics and telocentrics (a, t) as mono-armed. In order to homogeneously compare karyotype formulas, subtelocentric chromosomes in *M. nigricans*, previously classified as mono-armed by Vitturi (1992), were re-considered herein as bi-armed. Slides were processed for conventional staining with Giemsa; whereas CMA_3 and DAPI were used for GC- and AT-specific fluorochrome banding, respectively (Sumner, 1990).

FISH was performed on fixed spermatocyte chromosomes using three different probes: a sea urchin (*Paracentrotus lividus*, Echinodermata) 18S rDNA (partial sequence) (Cantone et al., 1993); a $(TTAGGG)_n$ vertebrate-type hexamer repeat; and the $(GATA)_n$ microsatellite. The latter two probes were obtained by PCR in the absence of template (Ijdo et al., 1991) using $(TTAGGG)_5/(CCCTAA)_5$ and $(GATA)_7/(TATC)_7$ as primers, respectively. Nick translation labelling of the 18S rDNA probe was performed with digoxigenin according to manufacturer's instructions (Roche). Telomeric and $(GATA)_n$ probes were DIG-labelled following the random priming protocol (Roche).

Slides were pre-treated with RNAse A (200 μg/ml) and then chromosomes were denatured in 70% formamide/2xSSC for 4 min at 72°C. Probes were denatured for 5 min at 80 °C. Each slide was hybridized with 25 μl of the hybridization mixture containing 120 ng of labelled and denatured probe dissolved in hybridization solution (50% formamide, 10% dextran sulphate, 2xSSC, 50 mM sodium phosphate). Hybridization reaction was allowed to proceed overnight in a moist plastic chamber at 37°C. Slides were washed at 42°C, mounted in propidium iodide + antifade solution (2 μg/ml) and viewed under a Leica filter set I3 (BP 450–490, LP 515) which allowed the simultaneous visualization of fluorescein-labelled hybrid (yellow) and chromosomal (red) DNA.

Nuclear AT-DNA content and haploid DNA content (C-value) were evaluated through flow cytometric assay performed on cell suspensions obtained from deep frozen digestive gland of *L. flavus* (N = 7), *T. sowerbyi* (N = 12), *D. panormitanum* (N = 6), and *M. nigricans* (N = 10) specimens. Peripheral blood erythrocytes from chicken (2C GS = 2.52 pg, 2C AT DNA = 1.39 pg; Ronchetti et al., 1995) were added to the slug cell suspensions as internal standard. Methods for sample preparation and nuclear DNA content evaluation were the same described in Vitturi et al. (2005). A BRITE-HS cytometer (Bio-Rad Laboratories) equipped with a xenon-mercury lamp was used for the analyses. Data were reported as mean ± standard deviation.

RESULTS

Chromosome Analysis

Haploid chromosome number (30 metaphase-I plates analysed per species) resulted n = 31 in *L. flavus* (Fig. 1A) and *D. panormitanum* (Fig. 1B) and n = 33 in *T. sowerbyi* (Fig. 1C).

Karyotypes obtained from five Giemsa-stained spermatogonial metaphases per species appeared highly symmetrical in the three taxa showing all bi-armed chromosome pairs. In particular, *L. flavus* consists of 15 metacentric, 12 submetacentric, and 4 subtelocentric pairs (Fig. 2A); *D. panormitanum*, of 12 metacentric, 13 submetacentric, and six subtelocentric pairs (Fig. 2B); and *T. sowerbyi* of 21 metacentric, 9 submetacentric, and three subtelocentric pairs (Fig. 2C).

Fluorochrome application was uninformative for detecting any repeated and/or compartmentalised DNA sequences. In fact, counterstaining of spermatogonial metaphases with CMA_3 and DAPI failed to give positive or negative fluorescence showing all chromosomes as homogeneously stained (Figs. 3A, 4A). Similarly, C-banding did not provide visible C-positive blocks (data not shown). In contrast, the $(GATA)_n$ probe showed abundant hybridization sites, dispersed as dot-like fluorescent yellow signals throughout all chromosomes (Fig. 5A–C).

Ribosomal probe labelled (in yellow) the terminal region of one chromosome pair in *L. flavus* (Fig. 6A) and *T. sowerbyi* (Fig. 6C) whereas, in *D. panormitanum* (Fig. 6B) hybridised with the termini of five chromosome pairs.

In the three species, the $(TTAGGG)_n$ sequence revealed typical labelled areas (in yellow) at the ends of all chromosomes (Fig. 7A–C).

Genome Size and DNA Base Composition

Mean C-values of GS and AT-DNA percentage in the total genome are summarized in Table 1.

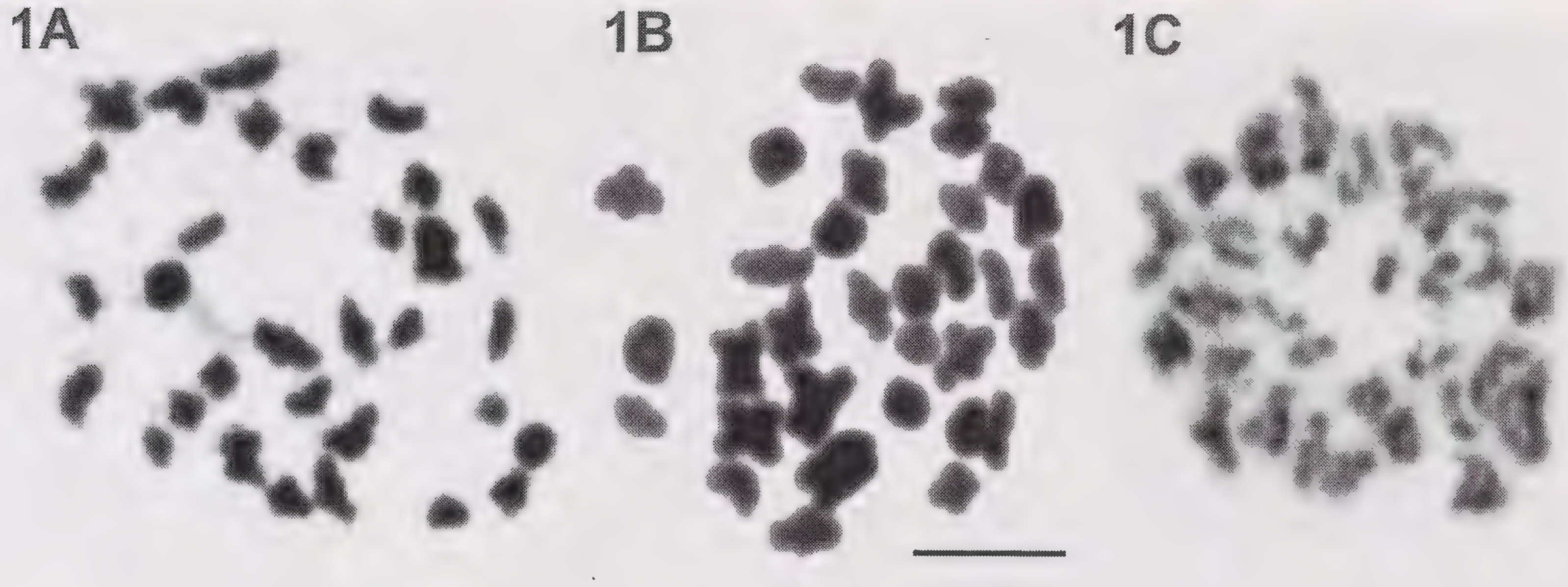


FIG. 1. Giemsa stained metaphase-I bivalents. 1A: *L. flavus*; 1B: *D. panormitanum*; 1C: *T. sowerbyi*. Scale bar = 10 µm.

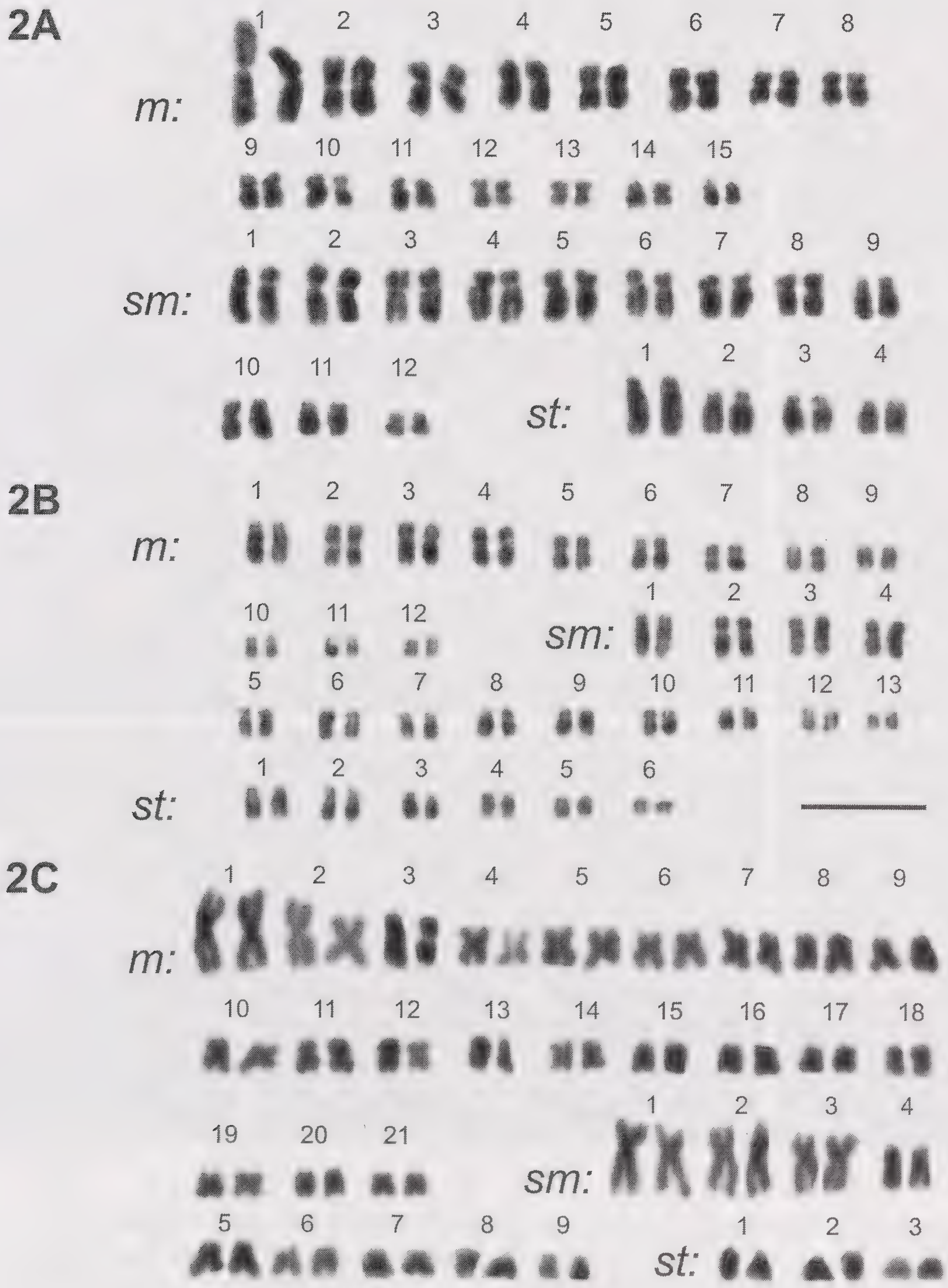
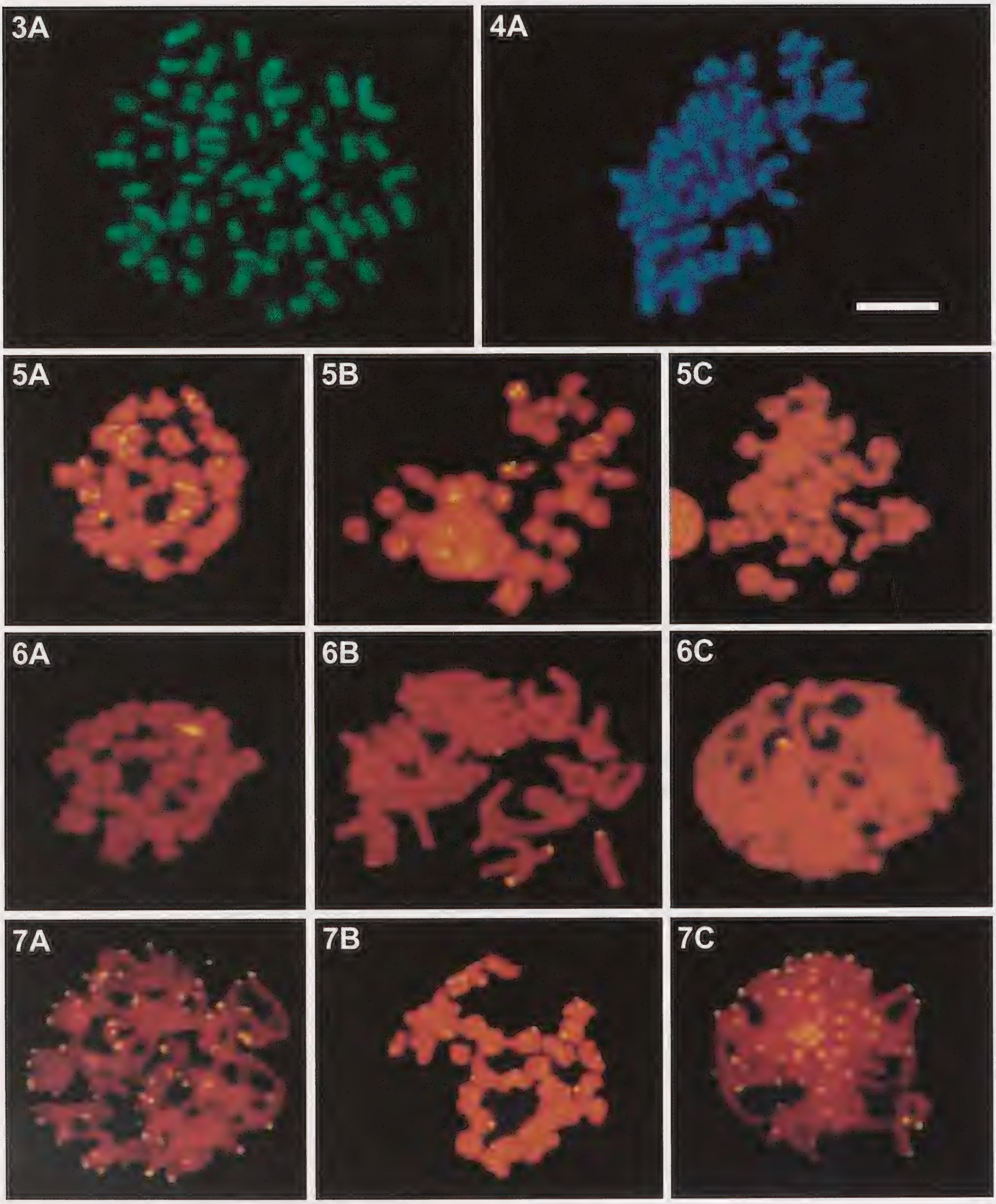


FIG. 2. Giemsa stained karyotype. 2A: *L. flavus* (2n = 62); 2B: *D. panormitanum* (2n = 62); 2C: *T. sowerbyi* (2n = 66). Chromosome pairs are shown clustered in three classes according to the chromosome morphology [metacentric (m), submetacentric (sm) and subtelocentric (st)] evaluated on the centromeric index. Scale bar = 10 µm.



FIGS. 3–7. Homogeneous staining patterns of spermatogonial metaphases of *L. flavus* with CMA_3 (green, 3A), and DAPI (blue, 4A) fluorochromes. Analogous dull fluorescence was observed in *D. panormitanum* and *T. sowerby* (data not shown); *in situ* hybridization patterns of the $(GATA)_n$ probe on spermatocyte chromosomes of *L. flavus* (5A); *D. panormitanum* (5B); and *T. sowerbyi* (5C); *in situ* hybridization with 18S rDNA (from *Paracentrotus lividus*) on spermatocyte chromosomes of *L. flavus* (6A); *D. panormitanum* (6B); and *T. sowerbyi* (6C). Yellow dots indicate where18S rDNA regions map on the chromosomes. $(TTAGGG)_n$ FISH patterns in *L. flavus* (7A); *D. panormitanum* (7B); and *T. sowerbyi* (7C). Scale bar = 10 μm.

TABLE 1. Compiled cytogenetical features in slug species of Milacidae, Limacidae, and Agriolimacidae (n.c. = not calculated).

Family	Species	n	2n	Karyotype formula	NOR number/ rDNA FISH	TTAGGG	Haploid DNA content (in pg) ± SD	AT %	References
Milacidae	*Milax gagates*	33 or 34					n.c.		Beeson, 1960
	Milax nigricans	33	66	32 (m + sm + st) + 1a			n.c.		Vitturi, 1992
	Milax nigricans	33	66		2	positive	n.c.		Vitturi et al., 2004
	Milax nigricans						2.34 ± 0.017	54.55	Present paper
	Tandonia sowerbyi	34					n.c.		Beeson, 1960
	Tandonia sowerbyi	33	66	21m + 9sm + 3st	2	positive	3.86 ± 0.049	53.85	Present paper
Limacidae	*Limax cinereoniger*	31					n.c.		Beeson, 1960
	Limax maximus	31					n.c.		Beeson, 1960
	Limacus flavus	31		15m + 12sm + 4st	2	positive	2.35 ± 0.014	53.43	Present paper
	Lehmannia marginata	24					n.c.		Beeson, 1960
	Lehmannia melitensis	20		20 (m+st)			n.c.		Vitturi & Sparacio, 1993
Agriolimacidae	*Deroceras agreste*	30					n.c.		Beeson, 1960
	Deroceras reticulatum	30					n.c.		Beeson, 1960
	Deroceras reticulatum						1.68	57.80	Vinogradov, 2000
	Deroceras panormitanum	31	62	12m + 13sm + 6st	10	positive	2.07 ± 0.014	49.17	Present paper

DISCUSSION

The three species show a substantial karyological homogeneity in (1) chromosome number and morphology; (2) fluorescent GC- or AT-specific staining patterns and (3) telomeric DNA composition. In fact, (1) their karyotypes share a haploid chromosome number (n = 31–33), which is common, although non exclusive, to the Limacidae, Agriolimacidae and Milacidae families (Patterson, 1969; Thiriot-Quievreux, 2003) and, as the majority of pulmonate species studied up to now, consist of all bi-armed elements; (2) both CMA_3 and DAPI dyes render all the chromosomes uniformly fluorescent; and (3) the hexamer $(TTAGGG)_n$ is the only motif observed in the telomeres.

Chromosome staining with Giemsa and fluorochromes were inadequate to reveal any inter-specific genomic difference, and the homogeneous DAPI and CMA_3 staining of chromatin indicated that GC- and AT-base pairs are equally interspersed throughout the genome. As previously reported for two Helicidae snail species of the genus *Cantareus* (Vitturi et al., 2005), CMA_3 was unable to identify the rDNA clusters. All the three species showed the $(TTAGGG)_n$ telomeric motif, as already reported for the other three investigated stylommatophoran species (Vitturi et al., 2004, 2005). This finding suggests that the “vertebrate” repeat $(TTAGGG)_n$ might be conserved within the order Stylommatophora. Conversely, a heterogeneous distribution of the vertebrate telomeric repeat has been already observed among Sacoglossa and Caenogastropoda since *Oxynoe olivacea* (Vitturi et al., 2000) and *Crepidula unguiformis* (Libertini et al., 2009) have a different telomeric motif although still unknown. Uniformity of Stylommatophora for the telomeric sequence can be clarified and resolved if more representatives species are analysed.

Karyological inter-specific heterogeneity becomes evident when the number of NOR sites and genome size (C-value) are compared. Major ribosomal sites are mapped at the terminal region in one chromosome pair (*L. flavus* and *T. sowerbyi*) or in five chromosome pairs (*D. panormitanum*). According to several authors, two NOR sites is presumed to be the plesiomorphic (ancestral) condition in vertebrates (Schmid et al., 1978) and invertebrates including molluscs (Pascoe et al., 1996; Thiriot-Quievreux, 2002; Vitturi et al., 2005). Taking into account this assumption, three out of four slug species analysed (Vitturi et al., 2004; present paper) seem to have retained the ancestral trait, whereas an increase in NOR number has occurred in *D. panormitanum,* probably through mechanisms of ribosomal cistrons translocation (Woznicki et al., 2000).

In spite of similarity in chromosome number, different genome sizes characterise the slugs of this study without showing any apparent evolutionary trend (Table 1). In fact, in *Tandonia sowerbyii* (Milacidae) GS amount (3.86 pg) is nearly the highest among Pulmonata (range 0.95–4.00 pg; Gregory, 2008), whereas in *D. panormitanum* (Agriolimacidae, 2.07 pg), *M. nigricans* (Milacidae, 2.34 pg), and *L. flavus* (Limacidae, 2.35 pg) GS values are similar and intermediate in the subclass range.

Observed inter-specific differences in GS values may reflect variations in highly and/or moderately repeated DNA quantities (Gregory, 2005). This assumption is not confirmed by C-banding which, in this case, failed to give any direct indication of constitutive heterochromatin. But, on the other hand, the presence of a high amount of $(GATA)_n$ DNA, would suggest that GS variation might depend on GATA-like short repeated sequences. A study on DNA contents in pulmonate gastropods (Vinogradov, 2000) reported on significantly higher GS and GC % (greater than 40%, i.e., corresponding to an AT-DNA percentage less than 60%) of terrestrial species as compared to related aquatic ones, suggesting as the most probable evolutionary trend a DNA accumulation accompanied by preferential increase in GC-fractions. Our data on GS and DNA base composition (Vitturi et al., 2005; present paper) confirm this tendency, which is consistent with Vinogradov’s assumption that high GS and GC% may be considered as an adaptive trait for terrestrial life conferring a greater physical stability to DNA by the triple H-bond of guanine-cytosine and by dispersion of this base pair in the genome.

In conclusion, present results are certainly an extension of cytogenetic data in pulmonates, but the characters found were not informative on phylogeny of Stylommatophora, being either symplesiomorphies – chromosome morphology and number, genome base pair composition; the occurrence of the $(TTAGGG)_n$ telomeric motif; two NORs – or autoapomorphies – five NOR-bearing chromosome pairs (*D. panormitanum*); a high GS value (*T. sowerbyi*). However, the potential of cytogenetic characters for phylogenetic reconstruction may be clarified and resolved if more representatives of each family are included in the analyses and, above all, if reliable synapomorphies are discovered.

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EFFECTIVE CONTROL OF INVASIVE APPLE SNAIL (*POMACEA CANALICULATA* LAMARCK) USING *PAECILOMYCES LILACINUS* (THOM) SAMSON

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INTRODUCTION

Apple snails of the genus *Pomacea* (Ampullariidae), a large, freshwater snail native to South America, were illegally introduced into Thailand to clean fish aquaria because of their ability to consume several kinds of aquatic plants and algae. The apple snail was first discovered in the wild in 1984 (Keawjam & Upatham, 1990). *Pomacea* in Thailand have been identified as *Pomacea canaliculata*, *Pomacea insularum*, and *Pomacea* sp. (Keawjam & Upathum, 1990), but Thaewnon-ngiw et al. (2004) indicated that the gene pool of *Pomacea* in Thailand is panmictic and should be recognized as a single species, *P. canaliculata*. The apple snails were discarded into waterways and rapidly spread out due to a few natural enemies, short maturation time, and potential to lay large numbers of eggs (Cowie, 2002). It has been reported to have caused serious damage to rice crops since 1996, and it is now regarded as being the most important rice pest in Thailand (Janyapeth & Archawakom, 1999; Chanyapate, 2004).

In Thailand molluscicides and insecticides have been used to control the apple snail, as in Taiwan, Japan, and the Philippines, without much success (Mochida, 1991). Some of these compounds have been shown to be extremely toxic to such non-target organisms as fish and other beneficial animals, and they present health risks to workers in the field (Anderson, 1993; Naylor, 1996). Niclosamide, endosulfan, camellia seed cake (residue), and copper sulfate, which are often used to control apple snail in Asian countries, cannot be registered in Japan because of their negative effects on the environment (Wada, 2004). Some botanical extracts have been tested, for instance, root of rotenone (*Derris elliptica*; Kardinan & Iskandar, 1997), but they are not as effective as saponin. However, all are highly toxic to fish (Suharto, 2000). Wu et al. (2005) reported on the successful use of morpholine, a chemical used as a waxing solvent on fruits to inhibit egg hatching. Recently, vulgarone B, a sesquiterpene from the plant *Artemisia douglasiana*, has reported molluscicidal activity against apple snail (Joshi et al., 2005). Laboratory bioassays indicated that vulgarone B had activity comparable with that of the commercial synthetic molluscicide metaldehyde. Conventional management practices, such as hand picking, fish (Halwart et al., 1996; Teo, 2006), and ducks (Teo, 2001) are labor intensive, uneconomical, and nonsustainable. For apple snail management, it is, therefore, imperative to develop an effective and practical method that is not harmful to human health and is environmentally friendly. An effective bacterial biological control agent has been reported by Chobchuenchom & Bhumiratana (2003). However, there are no published data on the use of fungi as a biological control agent for apple snails, whereas there are many publications reported in controlling insects (Sztejnberg et al., 2004; Fiedler & Sosnowska, 2007; Maketon et al., 2007, 2008; Steinkraus et al., 2002) and nematodes (Khan et al., 2004).

In this study, three entomopathogenic fungi and eight nematophagous fungi were screened for ovicidal activity on apple snail egg masses and molluscidal activity on newly hatched juveniles. The lethal concentration (LC_{50}) and time (LT_{50}) and enzymes production were evaluated for the most virulent fungus based on the hypothesis of enzymes hydrolysis assisting in fungal mycelial penetration through the egg and juvenile cell walls. Furthermore, it was subsequently formed into a wettable powder and tested for its efficacy as a microbial molluscicide in a greenhouse for understanding the full ramifications of such applications.

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MATERIAL AND METHODS

Isolation and Preparation of Conidial Suspension

Nematophagous fungi were isolated from a total of 60 soil samples obtained from various agricultural areas in Thailand. The isolation procedure was initiated by measuring 10 g soil mixed with 100 ml of sterile water in a beaker, with the addition of ten one-day-old apple snails. The number of snail deaths was observed daily for seven days. Death was determined by the lack of response when pricking the snails' head or foot. All dead snails were removed from the beakers, soaked in 10% sodium hypochlorite (Sigma-Aldrich, Missouri) for 5 min, and placed in a Petri dish containing Martin's Streptomycin Medium (Difco, Becton-Dickson, Sparks, Maryland). Fungi isolated were sent to Thailand Institute of Scientific and Technological Research (TISTR) for identification. In addition, three entomopathogenic fungi from our laboratory were used; they were *Metarhizium flavoviride* CKM-083 (Maketon et al., 2007), *Beauveria bassiana* CKB-048 (Maketon & Pitiporntapin, 2006), and *M. anisopliae* CKM-051 (Maketon et al., 2007).

All fungal isolates were grown on potato dextrose agar (Difco, Becton-Dickson, Sparks, Maryland) at 27 ± 1°C with 75–85% relative humidity in the room. The incubation period was 10–15 days. Conidial (spore) suspensions were made by lightly scraping the fungal culture surface with a sterile cell spreader into a sterile glass container. The conidial clumps were suspended in distilled water with 0.01% Tween 80 (ICI Americas, Norwich, New York). The suspensions were vortexed for 5 min to dissociate clumps and filtered through one layer of cheesecloth to remove conidial clumps and mycelial debris. Concentration of each suspension was diluted to 2×10^8 conidia/ml determined by a Neubauer hemocytometer under a phase-contrast microscope. The suspensions were used on the same day or the day after preparation and shaken before use. The pure fungal culture of the most virulent strain was deposited at TISTR.

Collection and Preparation of Apple Snails

All apple snails used in the laboratory experiment were collected using stick traps to obtain egg clutches around the pond at Kasetsart University, Bangkok. For ovicidal bioassay, egg clutches (one day after laying) were divided into 1 g each (approximately 200 eggs) using a brush pen, and placed on 9 cm diameter filter paper in a sterile Petri dish. For molluscidal bioassay, the egg clutches were placed above the waterline at room temperature (27 ± 1°C) until the young snails hatched and fell into the water. The water used for snail rearing was regular tap water left to stand for 24 h before use. Young snails were fed with rice leaves. In each test, ten snails of the same age group were sorted by size and placed into 9 cm diameter filter paper moistened with1 ml sterile water in a sterile Petri dish with a piece of rice leaf and replaced daily.

Experiment 1 – Fungal Virulence One Day after Egg Laying

Conidial suspensions of eleven fungi (Table 1) were sprayed on one day after laying apple snail eggs using a Thin Layer Chromatography (TLC) sprayer (Merck GmbH, Germany) at concentrations of 1 ml per gram of egg, resulting in approximately 1×10^8 conidia per gram of egg. Thirteen treatments with three replicates, including a water-treated and an untreated control, were performed. The percentage of unhatched apple snail eggs was recorded daily for 14 days after inoculation.

Experiment 2 – Two Most Virulent Fungi One, Five and Seven Days after Egg Laying

Conidial suspensions of the two most effective fungi from Experiment 1 (*Paecilomyces lilacinus* CKP-012 and *P. lilacinus* CKP-032) were prepared at the concentrations of 1×10^8 conidia/ml and then sprayed on one, five, and seven days after laying eggs using a TLC sprayer. Four treatments with five replicates, including a water-treated and an untreated control were performed. The percentage of unhatched eggs was recorded daily for 14 days after inoculation.

Experiment 3 – Two Most Virulent Fungi on One-, Five-, and Ten-Day-Old Juveniles

The experiment was performed exactly the same as Experiment 2, except with test subjects were one-, five-, and ten-day-old juveniles.

Experiment 4 – Lethal Concentration (LC_{50}) and Lethal Time (LT_{50})

For $LC_{50,}$ conidial suspensions of the most virulent fungus from Experiment 1 were prepared at concentrations ranging from $1x10^6$ to $1x10^9$ conidia/ml and tested on one and five days after laying eggs, and one and five-day-old juveniles. Mortality was checked daily for five days. Similar procedures were followed for LT_{50}, except only one conidial suspension concentration of $1x10^8$ conidia/ml was sprayed on eggs and juveniles. Probit analysis (Finney, 1971) was used for calculation with 95% confidence intervals.

Experiment 5 – Enzymes Produced by the Most Virulent Fungus

Methods of Khan et al. (2003) and Dackman et al. (1989) were employed for protease determination. Skimmed milk (0.2% W/V) was added to minimal medium (Bonants et al., 1995) cultures in 1-liter conical flasks containing 250 ml culture medium and inoculated with the most virulent fungus from Experiment 1 at $1.0x10^6$ conidia/ml. Cultures were incubated on a shaker at 125 rpm for seven days at 27°C. Samples were drawn daily and diluted in a carbonate-bicarbonate buffer pH 10.2 (Dawson et al., 1986). One milliliter of diluted sample was incubated with 1 ml of 1% azocasein at 50°C for 30 min. The reaction was terminated by the addition of 1.5 ml 5% trichloroacetic acid. Non-igested azocasein was separated by centrifuging at 600 x g for 20 min and culture supernatants filtered through 0.2 µm membranes. Protease activity was measured at 345 nm and expressed as dry weight (mg/ml) of azocasein solubilized obtained from the formula A_{345} x 0.566 x dilution factor as described (adapted from Khan et al., 2003).

For chitinase production, minimal medium was supplemented with 0.5% (W/V) chitin. One-liter conical flasks containing 250 ml of medium were inoculated with $1.0x10^6$ conidia/ml of the most virulent fungus and incubated at 125 rpm for 7 days at 27°C. Similar to protease detection, 1.5 ml of sample were drawn daily and incubated with 1ml of 1% colloidal chitin at 37°C for 2 h (adapted from Dackman et al., 1989; Khan et al., 2003). Precipitate was separated, and the supernatant was measured at 285 nm. Chitinase activity was indicated by the increase in absorbance of N-acetyl-L-glucosamine.

The method of Miller (1959) was modified for measuring the amount of glucose released after an amylase enzyme hydrolysis using starch as a substrate. The reagents used were 1% 3,5-dinitrosalicylic acid, 30% potassium sodium tartrate (Rochelle salt), 0.05% sodium sulfite, and 1% sodium hydroxide. The substrate, reagents, and mycelial-free supernatant mixture were incubated at 37°C for 10 min, and then the change in absorbance at 575 nm was measured.

Lipase activity was estimated following Lee et al. (1999) using a spectrophotometric assay with p-nitrophenyl butyrate as a substrate, which was dissolved in acetonitrile at a concentration of 10 mM. Subsequently, ethanol and 50 mM potassium phosphate buffer (pH 7.5) were added to a final composition of 1:4:95 (v/v/v) of acetonitrile/ethanol/buffer, respectively. The mycelial-free supernatant (0.3 ml) was added to the substrate solution (0.9 ml), and the mixture was incubated at 60°C. After 15 min, enzyme activity was measured by monitoring the change in absorbance at 450 nm, which represents the amount of released p-nitrophenol. All chemicals used were from Sigma-Aldrich, Missouri.

Experiment 6 – Greenhouse Tests

Preparation of Apple Snail Eggs on Rice Stalks: Fifty pairs of adult apple snails were collected from the campus ponds of Kasetsart University, Bangkok, and placed in 20 round containers (50 cm diameter, 30 cm height) with planted rice stalks (strain Tai Chung Native 1) in a greenhouse, where they laid their eggs, two egg masses per container were used.

Preparation of Conidia into a Powder Form: The two most effective fungi from Experiment 1 were suspended in a 3% Tween 80® wetting agent at a final concentration of $1x10^9$ conidia/g, and freeze dried using sterile attapulgite clay (AGSORB-325® LVM-GA, OIL-DRI Corp., IL) as a filler in a Heto FD3 (Denmark) freeze dryer.

Two Best Fungi Compare to Chemicals on Apple Snail Eggs: A chemical molluscicide, niclosamide 70% WP (Bayluscide™, Bayer Cropsciences, Germany) was used as a comparison. Conidial suspensions from both fungal powders were prepared and sprayed on eggs which resulted in approximately $3.33x10^7$

TABLE 1. Percent hatched of one-day-old apple snail eggs after 14 days. Means followed by the same letter in the same column showed no significant difference at 95% by DMRT.

Fungal isolate	Percent hatched		
	7 days after inoculation	10 days after inoculation	14 days after inoculation
Beauveria bassiana CKB-048	76.35 ± 6.70^{ab}	85.50 ± 4.16^{a}	90.05 ± 8.77^{a}
Metarhizium anisopliae CKM-051	72.42 ± 7.24^{b}	78.83 ± 6.14^{b}	83.57 ± 6.27^{b}
M. flavoviride CKM-083	80.06 ± 2.13^{a}	86.70 ± 5.65^{a}	87.77 ± 7.89^{ab}
Paecilomyces lilacinus CKP-012	8.53 ± 2.21^{e}	10.50 ± 4.10^{f}	12.22 ± 8.37^{g}
P. lilacinus CKP-032	10.90 ± 4.63^{e}	16.23 ± 5.05^{f}	20.43 ± 6.63^{f}
P. lilacinus CKP-073	26.23 ± 8.16^{c}	39.94 ± 6.74^{d}	49.22 ± 8.23^{d}
P. lilacinus CKP-070	25.40 ± 3.13^{c}	40.00 ± 1.25^{d}	46.00 ± 6.80^{d}
P. lilacinus CKP-063	20.75 ± 8.27^{d}	29.53 ± 8.26^{e}	34.15 ± 6.86^{e}
P. lilacinus CKP-083	22.24 ± 7.14^{d}	32.20 ± 2.76^{de}	36.47 ± 6.76^{e}
P. lilacinus CKP-093	12.60 ± 2.40^{e}	19.60 ± 8.41^{f}	23.60 ± 7.15^{f}
P. lilacinus CKP-004	30.40 ± 6.76^{c}	52.86 ± 7.10^{c}	63.40 ± 7.24^{c}
Control (untreated)	92.20 ± 4.10^{a}	88.18 ± 6.40^{a}	95.25 ± 3.20^{a}
Control (water treated)	91.80 ± 6.70^{a}	90.10 ± 5.44^{a}	91.04 ± 4.60^{a}
% C.V.	72.03	56.53	51.54

conidia per g egg. Concentration of the known and commercially available molluscicide was 3.50 mg a.i. of niclosamide 70% WP and was sprayed on each egg mass. Four plastic boards 60×60 cm in size were used to prevent aerosol migration at each container while spraying.

Experiment 7 – Effect of Clay and Wetting Agent to One-Day-Old Eggs and Juveniles

The effects of clay and wetting agents used as fillers from Experiment 6 were tested. The sample was prepared as in Experiment 6, except no fungal spore was added, and water was sprayed for a comparison. The percent of one-day-old eggs hatched and the percent of mortality of one-day-old juveniles was recorded daily for 14 days after sprayed.

Statistical Analysis

Duncan's new multiple range test (DMRT) was used for calculation and interpretation of statistical difference among treatments.

RESULTS

Eleven fungal isolates exhibited larvicidal capability and were identified according to standard mycological characterization (Domsch et al., 1993) and polymerase chain reaction techniques (Inglis et al., 2005), but there were only two isolates that showed promising results; they were *Paecilomyces lilacinus*: CKP-012 and CKP-032.

Experiment 1 – Fungal Virulence One Day after Egg Laying

The apple snail eggs hatched 7–12 days after deposition. The eggs in the water treated and untreated control treatments started hatching seven days after deposition with 91.04 ± 4.6 and 95.25 ± 3.2% of success (Table 1) (means ± SE). The eggs treated with doses of *M. flavoviride* CKM-083, *B. bassiana* CKB-048, and *M. anisopliae* CKM-051 showed no difference in rates of hatching within the same time frame, and these three isolates were considered ineffective strains. Comparatively, treatments with all eight strains of *P. lilacinus* resulted in delayed egg hatching. Egg hatching percentages at 10 days after inoculation showed similar results to the seven days post-inoculation results. For all eggs infected by isolates of *P. lilacinus* again appeared to have low hatching percentages; the highest hatching rate was still found in CKP-004, followed with CKP-070, CKP-073, CKP-083, CKP-063, CKP-093, CKP-

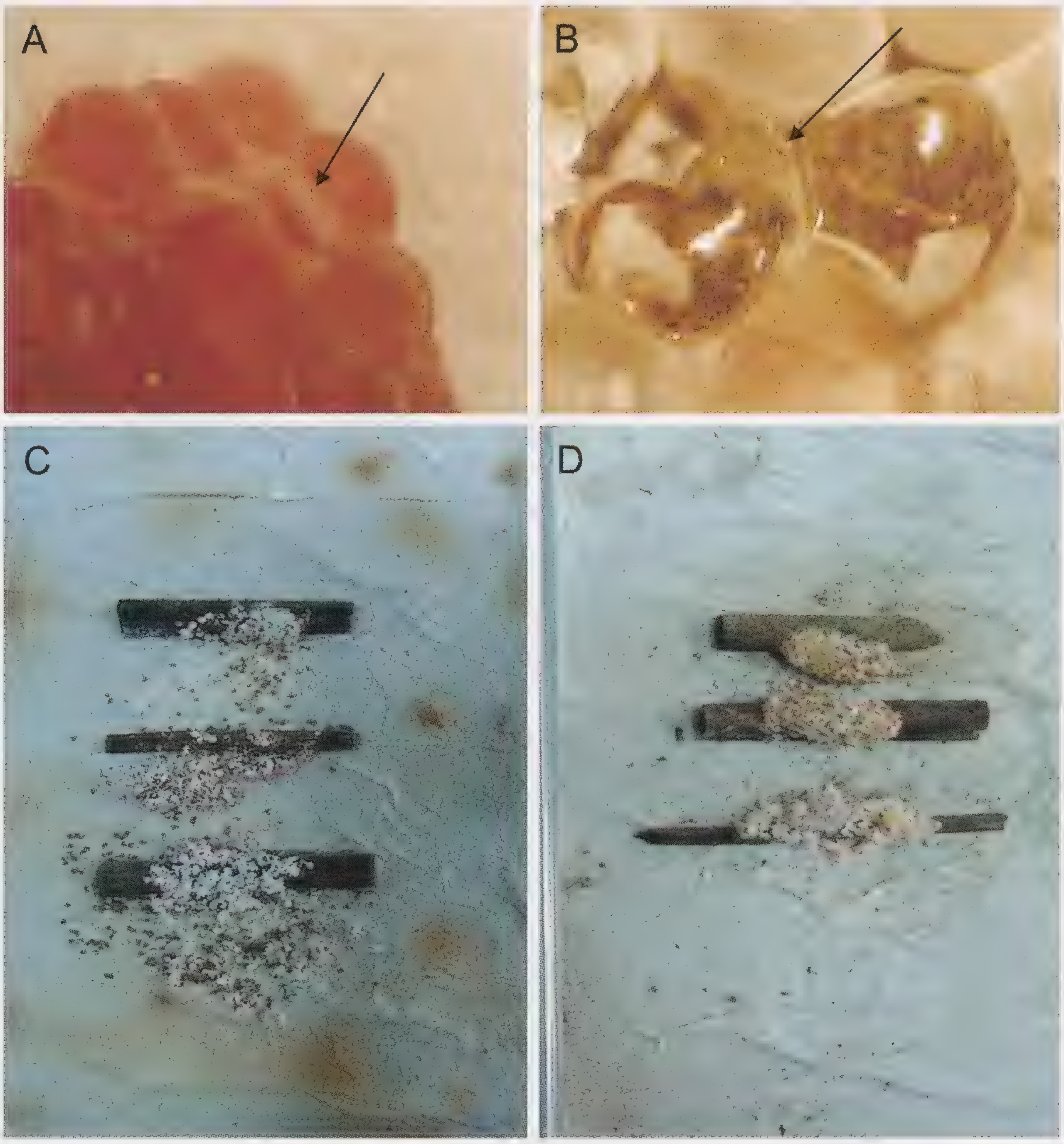

FIG. 1. Apple snail eggs infected by *Paecilomyces lilacinus* CKP-012 showing fungal mycelium development after 4 days (A) and some eggs shell were hydrolyzed after 10 days (B), and most eggs become juveniles from the water treated control after 14 days (C) while only a few hatched from *P. lilacinus* CKP-012 treated after the same period (D).

032, and CKP-012, respectively. Mycelium of CKP-012 appeared on eggs after four days, and some egg shells were hydrolyzed after ten days (Fig. 1).

At 14 days after inoculation, similar results were observed for the high mortality of developing juveniles from the two most effective *P. lilacinus* which were CKP-012 followed by CKP-032.

Experiment 2 – Two Most Virulent Fungi One, Five and Seven Days after Egg Laying

After having screened for the most successive ovicidal behavior, two *P. lilacinus* isolates, CKP-012 and CKP-032, were further studied for their efficacies in controlling different developmental egg stages. After 14 days, CKP-012 was superior to CKP-032. The percent hatched were 13.67 ± 3.43, 17.57 ± 4.16, and 20.55 ± 6.76 for CKP-012 treated at one, five, and seven-day-old eggs, respectively. Also, 19.12 ± 5.61, 24.17 ± 3.12, and 31.22 ± 2.11% hatched from CKP-032 treated at one, five, and seven-day-old eggs, respectively (Fig. 2A). While in the water treated and untreated controls, most eggs become juveniles with only 3 to 6% of the juveniles dying.

Experiment 3 – Two Most Virulent Fungi on One-, Five-, and Ten-Day-Old Juveniles

After two weeks, *P. lilacinus* CKP-012 had a high capability of killing newly hatched juveniles; however, with increasing maturity, juveniles became more tolerant to the fatal effects of the fungi. *P. lilacinus* CKP-012, which resulted in higher mortality than CKP-032. The

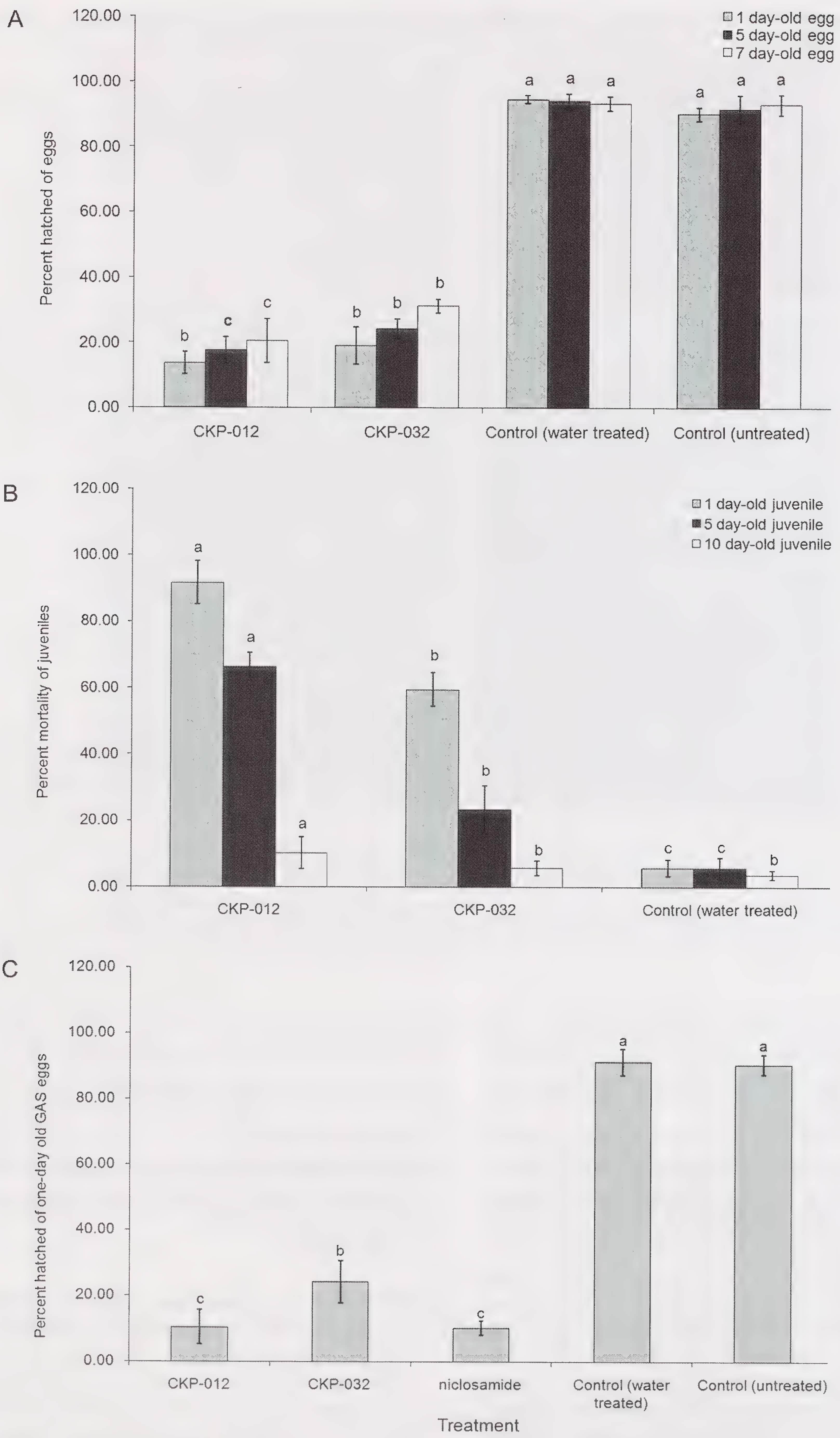

FIG. 2. Percent hatched of apple snail eggs after 14 days. (A) Percent mortality of apple snail juveniles after 14 days; (B) and Percent hatched of one-day-old apple snail eggs after 14 days; (C) Means followed by the same alphabet on the same day showed no significant difference at 95% by DMRT.

TABLE 2. Enzymes production from *Paecilomyces lilacinus* CKP-012.

Day	Protease activity[a]	Chitinase activity[b]	Amylase activity[c]	Lipase activity[d]
1	0.0000	0.0779	0.3140	0.1010
2	0.0000	0.0808	0.3080	0.1040
3	1.6298	0.0869	0.3370	0.0980
4	1.1507	0.0989	0.2970	0.1090
5	1.4787	0.0966	0.1090	0.1610
6	2.7219	0.0954	0.0920	0.2800
7	2.7318	0.1000	0.0950	0.2870
Control	0.0000	0.0000	0.0000	0.0000

[a] Protease activity was measured as dry weight (mg/ml) of azocasein solubilized = A_{345} x 0.566 x dilution of the sample. Protease and azocasein mixture was incubated at 50°C and pH 10.2 for 30 min.

[b] Chitinase activity was measured by the increase in absorbance of N-acetyl-L-glucosamine at 285 nm. Chitinase and chitin mixture was incubated at 37°C and pH 5.1 for 2 h.

[c] Amylase activity was measured as 1 µmole of D-Glucose released from starch solution per min at 37°C for 10 min.

[d] Lipase activity was measured as 1 µmole of p-nitrophenol released from p-nitrophenyl butyrate per min at 60°C for 15 min.

percentages of mortality for one-, five-, and ten-day-old juveniles treated with CKP-012 were 91.67 ± 6.50, 66.32 ± 4.21, and 10.20 ± 4.73, respectively; while those treated with CKP-032 were 59.50 ± 5.10, 23.30 ± 7.30, and 5.70 ± 2.14%, respectively (Fig. 2B). Dehydration in the untreated control detrimentally affected the test because young juveniles started dying after four days and were decimated after nine days.

Experiment 4 – Lethal Concentration (LC_{50}) and Lethal Time (LT_{50})

The level of lethal concentration required for *P. lilacinus* CKP-012 in preventing hatching of 50% apple snail eggs was 8.63×10^7 conidia/ml for one-day-old eggs and 1.58×10^8 conidia/ml for five-day-old eggs. While the LC_{50} for killing one-day-old and five-day-old juveniles were 1.74×10^8 and 3.71×10^8 conidia/ml respectively. The lethal time required to inhibit the hatching of 50% of the eggs treated after one day was 2.26 days and 4.36 days for those treated on day five. However, longer times were required for killing juveniles at 3.43 days and 4.38 days for one-day-old and five-day-old juveniles respectively.

Experiment 5 – Enzymes Produced by the Most Virulent Fungus

Quantitative study showed that *P. lilacinus* CKP-012 produced four types of enzymes; they are a protein, a chitin, a carbohydrate, and a lipid digesting enzymes (Table 2). The concentration of four digesting enzymes based on a seven-day trial. The results show that protease, chitinase, and lipase activities tend to increase significantly from day one to day seven. Protease activity increased from 0.000 to 2.7318, chitinase activity increased from 0.0779 to 0.1000, and lipase activity increased from 0.1010 to 0.2870. However, amylase activity decreased from 0.3140 to 0.0950; this might have resulted from the fact that starch was not an appropriate substrate for the specific amylase produced.

Experiment 6 – Greenhouse Tests

Results of a comparative test of two strains of *P. lilacinus*, CPK-012 and CPK-032, against a known chemical molluscicide (niclosamide) are shown in Figure 2c. After 14 days, there were no significant difference in the hatching percentage of one-day-old eggs treated with CKP-012 and niclosamide 70% WP, which were 10.50 ± 5.12 and 10.27 ± 2.10, respectively ($p > 0.05$). However, both treatments were significantly more effective than *P. lilacinus* CKP-032, which hatched at 28.22 ± 6.40% while the water's treated and untreated controls had 91.25 ± 4.10 and 90.50 ± 3.10% hatched, respectively. Developing juveniles in the greenhouse were difficult to observe because newly hatched juveniles immediately migrated into the water and were inaccessible. However, a repellent effect from fungal application was noted because a number of newly introduced female apple snails would not lay their eggs on previously treated rice stalks. New rice stalks had to be replaced before egg laying could be initiated.

Experiment 7 – Effect of Clay and Wetting Agent to One-Day-Old Eggs and Juveniles

Clay and wetting agents used as fillers had negligible effects on one-day-old eggs, with the percent hatching of 88.86 ± 1.93 and 89.26 ± 2.64 that occurred from water-treated and filler treated, respectively. A similar trend happened in juveniles; the mortality was quite low with 10.00 ± 0.72 and 11.67 ± 0.84 occurring in water-treated and filler treated specimens, respectively.

DISCUSSION

Samson (1974) classified *Paecilomyces lilacinus* as a fungus used for controlling some kind of nematodes that cause plant diseases (Jatala, 1986). Those nematodes mainly belong to the genus *Meloidogyne*, which causes root-knot symptoms in some agricultural plants. Nematophagous fungi penetrate the host cell by means of mechanical pressure as well as enzymatic digestion (Dackman et al., 1989; Morgan et al., 1983; Stirling, 1991). Bonants et al. (1995) found that *P. lilacinus* (CBS 143.75) can produce protease to alter the embryogenesis of the eggs of *Meloidogyne incognita*, essentially stopping the hatching of juveniles. Results from our work showed that *P. lilacinus* CKP-012 can also grow into apple snail egg by penetration through the egg wall and establishing a mycelium in the egg matrix. The mechanism might have occurred from fungal enzymes' hydrolysis on the egg shells, encouraging the mycelium to penetrate because the egg shells not only consist of calcium, but also such biochemical substances as caroteno-protein complexes (Heras et al., 2007). Therefore, other constituents are amenable for enzyme hydrolysis, especially protease and lipase. From this study, the protease content excreted by the fungus was high enough to lyse the protein matrix on the egg shell. For lipase, even though its content was fairly low, it still had an effect in digesting the lipid portion, in this case the carotenoid moiety. Once the fungal mycelium passes through the shell, its enzymes will continue to play an important role in digesting several biochemical constituents in the perivitelline fluid. According to Heras et al. (1998), biochemical composition at stage I is represented by carbohydrates, proteins, and lipids with 34.8%, 13.0%, and 1.5% dry weight, respectively; these are the major source of nutrients for the embryo. The carbohydrate moieties were not only monosaccharide, which include mannose and galactose, but also N-acetylglucosamine, a chitin precursor (Heras et al., 2007). Thus, a chitinase enzyme may also play an additional role in digesting or inhibiting a complete ovorubin formation. This effect was similar to that found by Khan et al. (2004), who studied the effect of *P. lilacinus* on eggshell of *M. javanica*, the fungus showed its efficacy in disintegration of vitelline, chitin, and lipid layers of *M. javanica*'s eggs. Vitelline and lipid layers support eggs by providing structural uniformity and permeability characteristics, respectively. Damage to these two layers caused by enzyme treatment enabled other metabolites to permeate the eggs causing changes such as swelling of eggs. Therefore, enzymes may facilitate physical penetration and improve efficiency of infection.

P. lilacinus CKP-012 had a high capability for killing newly hatched juveniles; however, with increasing maturity, higher tolerance appeared to the fatal effects of the fungus. This could result from the fact that the snail's shell becomes thicker and possibly more difficult to penetrate. However, from this study, *P. lilacinus* demonstrated promising results as a biocontrol agent for combating the apple snails, especially when applied to egg stage, *P. lilacinus* performed as effectively as a commercial chemical based molluscicidal agent. Therefore, *P. lilacinus* should be considered as a biological control agent for controlling apple snail in an integrated pest management program.

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INFLUENCE OF TEMPERATURE, SIZE AND SEX ON AERIAL RESPIRATION OF *POMACEA CANALICULATA* (GASTROPODA: AMPULLARIIDAE) FROM SOUTHERN PAMPAS, ARGENTINA

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INTRODUCTION

Pomacea canaliculata (Lamarck 1822) is a freshwater snail belonging to the family Ampullariidae, whose natural distribution comprises most of the Plata river Basin and extends southwards to Tandilia and Ventania mountains in southern Pampas, Buenos Aires Province, Argentina (Martín et al., 2001). International concern about this voracious macrophytophagous snail rose when it, and other related species, became established as a serious pest of aquatic crops, including rice and taro, in South and East Asia (Cowie et al., 2006). In addition, the role of invading ampullariids as promoters of ecosystemic changes in invaded natural wetlands has been recently highlighted (Carlsson et al., 2004). Nowadays, *P. canaliculata* is listed among the top 100 worst invasive alien species of the world (Lowe et al., 2000), being the only freshwater snail included and probably the species with the greater potential for spread in North America (Rawlings et al., 2007).

Ampullariids or apple snails have both a well-developed gill and a pulmonary sac or lung, and are commonly considered as "amphibious" snails (Andrews, 1965; Berthold, 1991). Water flows inside the pallial cavity through a furrow-shaped left nuchal lobe and leaves it through the right nuchal lobe. The lung is located in the left side of the mantle roof and opens anterolaterally to the pallial cavity. In *Pomacea,* during aerial respiration, the left nuchal lobe lengthens and rolls up to form a tube-shaped siphon, which conveys air into the lung, allowing the snails to ventilate it without reaching the water surface. During lung ventilation, pumping movements produced by the partial retraction of the cephalopodium generates a pressure differential leading to the exchange of air. The use of lung's air as a physical gill has been denied (Andrews, 1965), although it increases apple snail's buoyancy (Burky & Burky, 1977; Dillon, 2000).

Lung ventilation frequency in the few *Pomacea* species studied increases with water temperature (McClary, 1964) and decreases with body size, both in intra- and interspecific comparisons (Burky & Burky, 1977), but the basic parameters of aerial respiration are unknown for *P. canaliculata*. Most previous studies refer to species with tropical and subtropical ranges of distribution. However, *P. canaliculata* inhabits subtropical and temperate regions and remains active at low temperatures that are lethal or induce lethargy in the remaining species (Cowie, 2002). *Pomacea canaliculata* also shows considerable sexual dimorphism in such traits as shell and operculum shape (Estebenet et al., 2006), longevity, growth rate (Estebenet & Martín, 2002; Martín & Estebenet, 2002), and midgut gland size (Vega et al., 2005). These differences suggest the possibility of intersexual differences in metabolic rates and/or respiratory organs and hence on aerial respiratory behaviour.

Pomacea canaliculata deposit egg masses above the waterline, and the embryos fully develop inside the cleidoic eggs (Pizani et al., 2005). During development, the embryos are submersed in a perivitelline fluid providing them with structural precursors and energy supplies (Heras et al., 1998) until eggs hatch by mechanical fracture of the calcareous egg shell. It seems highly improbable that the embryos can fill the lungs except at the final stages of development when the perivitelline fluid has been totally incorporated. However, it is unknown whether lung ventilation begins just before falling from the egg mass or, alternatively, how long after the snails have fallen into the water.

Aerial respiration allows *Pomacea* spp. to thrive in freshwater habitats with poorly oxygenated waters and to endure dry-down conditions (Andrews, 1965; Cowie, 2002; Yusa et al., 2006). However, a number of drawbacks to the use of atmospheric oxygen has been suggested, such as limitations in distribution due to dependence on air access (Darby et al., 2002) and a higher vulnerability to specialized visual predators such as the snail kite *Rostrhamus sociabilis* (Vieillot, 1817) (Bennetts et al., 1994; Stevens et al., 2002). To better understand the role of aerial respiration on the ecology and behaviour of *P. canaliculata*, we analyzed the effect of temperature, size and sex on lung ventilation frequency and duration and number of pumping movements in this species.

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MATERIALS AND METHODS

Snails used in this study were hatched from eggs laid in the laboratory by mature snails obtained in the summer 2004 from a population in Curamalal stream (37°14'31"S, 62°08'04"W, Buenos Aires Province, Argentina), a watercourse originated in the Northern slope of Ventania Mountains. This stream section is narrow (3–8 m width) and shallow (mean depth less than 75 cm), although in some narrow places depth can reach up to 1.5 m; water velocities range from 0.39 to 0.93 $m.s^{-1}$ during normal discharge conditions. Patterns of daily and seasonal variation in water temperature in Curamalal stream were obtained from hourly readings of a submersible data-logger anchored to the bottom (1.2 m depth) during an entire year. The snails were maintained in aquaria with $CaCO_3$ saturated tap water in a rearing room at 25 ± 2°C, under 14 h light/ day and fed with lettuce *ad libitum* (Martín & Estebenet, 2002).

Laboratory trials to estimate the parameters of aerial respiration were performed in 24 l glass aquaria (20 cm-wide, 40 cm-long and 30 cm-deep) with a sand layer deep enough to allow the snails to bury themselves (3 cm). Water temperatures (T) were kept constant at five different levels (10, 15, 20, 25 and 30°C), with electric thermostats located in the bottom of each aquarium to avoid thermal stratification. Aquarium water was continuously aerated with electric air pumps provided with diffusers in order to maintain dissolved oxygen at saturation levels. Snails used in the trials ranged from 13.5 to 47.0 mm of total shell length (L, from the apex to the extreme lip of the aperture, measured with a Vernier calliper to the nearest 0.1 mm). Snails in which the testicle was observed through the translucent shell (Takeda, 1999) or with a humped operculum (Estebenet et al., 2006) were considered to be males. Snails without these male traits and smaller than 25 mm were considered as sexually undifferentiated, and those larger than that size as females, since a minimum of 25 mm has been considered necessary for maturation in females (Estebenet & Martín, 2002).

For each trial, six snails were selected to cover uniformly the whole size range and individually marked with synthetic polish. The snails were subjected to an acclimation period of 72–96 h, during which the water temperature was changed at a rate of 1°C per hour from 25°C to the desired one in the first hours; lettuce was supplied to each aquarium at a maximum rate of one leaf a day.

Snails were observed continuously for two hours in order to register respiratory activities. Thirty minutes before each observation period, lettuce remains were carefully removed and a new whole leaf was anchored to the bottom to avoid the continuous permanence of the snails at the waterline and to facilitate the register of siphonal movements. Just before each observation period water temperature was recorded and the air pumping system was disconnected to avoid interference with the observations. None of these procedures elicited negative responses (such as sinking, withdrawal, siphon retraction) in the snails. The starting and ending times and the number of pumping movements per lung ventilation during the observation period were recorded for each snail. The scheme was repeated three times for the 10°C and 15°C treatments and six times for the others. One hundred and forty-four snails were used in the trials, and each snail was used only once.

On the basis of the total records during the observation period the following variables were calculated: lung ventilation frequency ($VF(h^{-1})$ = number of lung ventilations / 2 h), number of pumping movements per lung ventilation (NP = number of pumping movements / number of lung ventilations) and duration of each pumping movement (DP(s) = time ventilating / number of pumping movements). Except for VF, only snails that ventilated at least once were included in the calculations.

Reliable measurements of siphon length proved to be impracticable due to its constant change in shape and position. As a surrogate, snail depth during lung ventilation was determined in a second group of trials, performed in the same 24 l aquaria with six snails of sizes ranging from 15.1 to 53.3 mm and an acclimation period of 24 h. In order to maximize lung

TABLE 1. Summary of two-way ANOVAs for the aerial respiration variables of *Pomacea canaliculata* (for differentiated snails only). Dependent variables (square root-transformed): lung ventilation frequency (VF), number of pumping movements per lung ventilation (NP) and duration of each pumping movement (DP); fixed factors: T (temperature), S (sex) and interaction (T x S).

Variables	MS error	Sex	Temperature	T x S
VF	0.5609	$F_{1,76} = 2.156$ $P = 0.1462$	$F_{4,76} = 32.081$ $p < 0.0001$	$F_{4,76} = 0.265$ $P = 0.8997$
NP	0.1535	$F_{1,50} = 0.014$ $P = 0.9060$	$F_{3,50} = 0.896$ $p = 0.4498$	$F_{3,50} = 1.289$ $P = 0.2883$
DP	0.0041	$F_{1,50} = 2.011$ $P = 0.1624$	$F_{3,50} = 145.147$ $p < 0.0001$	$F_{3,50} = 7.101$ $P < 0.0001$

ventilation frequency, water temperature was maintained at 30°C and no artificial aeration was provided. The distance from the water surface to the nearest point of the shell aperture at the beginning of a lung ventilation was recorded for 225 snails (those used in the previous trials plus an extra group from the same laboratory stock) while they were attached to the lateral walls of the aquarium; in most cases (93%) the depth of five to seven lung ventilations was registered for each snail, and maximum depth (MD (mm)) and average depth (AD (mm)) were calculated.

Eight hundred and seventy-two hatchlings obtained from four egg masses laid by laboratory snails on the aquarium walls were observed under a stereoscopic microscope at room temperatures (20–25°C) to investigate the presence of air in the lung and the respiratory behaviour. Ready to hatch eggs from the outer layer of the aerial egg masses were dissected with the same purpose, tearing apart the calcareous egg shell with a needle.

To test for the effect of temperature, sex and state of sexual differentiation over the aerial respiration variables, two-way ANOVAs were performed after a square root transformation as suggested by the Box-Cox method (Box & Cox, 1964). Analyses of differences among males, females and undifferentiated snails in the average and maximum distance during lung ventilation, were carried out with one-way ANCOVAs using length as covariate, after a log-log transformation.

RESULTS

Water temperatures in the Curamalal stream fluctuated greatly within the year, ranging from 31.5°C in January to a minimum of 0.3°C in July. Daily fluctuations were high, with a maximum recorded variation in one day of 17.8°C during December. Instantaneous temperature records showed nearly the same values along all depths.

TABLE 2. Summary of two-way ANOVAs for the aerial respiration variables of *Pomacea canaliculata* (for all snails; males and females not pooled for DP analysis). Dependent variables (square root-transformed): lung ventilation frequency (VF), number of pumping movements per lung ventilation (NP) and duration of each pumping movement (DP); fixed factors: T (temperature), D/S (state of differentiation or sex) and interaction (T x D/S).

Variables	MS error	Differentiation/Sex	Temperature	T x D/S
VF	0.5281	$F_{1,132} = 5.625$ $p = 0.0192$	$F_{4,132} = 38.782$ $p < 0.0001$	$F_{4,132} = 1.133$ $p = 0.3436$
NP	0.1670	$F_{1,84} = 17.246$ $p < 0.0001$	$F_{3,84} = 0.338$ $p = 0.7977$	$F_{3,84} = 1.634$ $p = 0.1877$
DP	0.0045	$F_{2,80} = 2.1002$ $p = 0.1291$	$F_{3,80} = 217.9807$ $p < 0.0001$	$F_{6,80} = 4.2099$ $p = 0.0009$

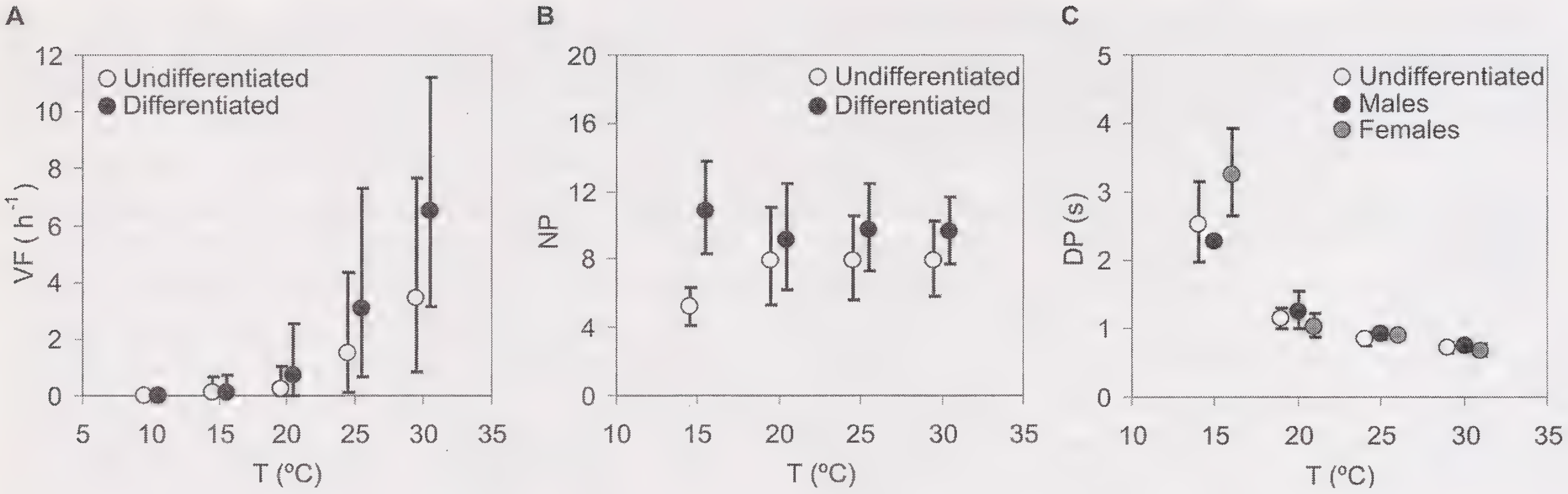

FIG. 1. Means ± SD (back-transformed) of aerial respiration variables of undifferentiated and differentiated *Pomacea canaliculata* snails vs. temperature (T): A) lung ventilation frequency (VF), B) number of pumping movements per lung ventilation (NP) and C) duration of each pumping movement (DP, males and females not pooled).

No significant differences were found between males (n = 41) and females (n = 45) for any of the aerial respiration variables (Table 1), although a significant interaction was detected for DP; accordingly, except for DP, both sexes were pooled in a single category (sexually differentiated) to compare them with the undifferentiated snails (n = 56) at the different temperatures tested. Lung ventilation frequency showed a positive significant effect of temperature and sexual differentiation; the undifferentiated snails ventilated significantly less often (64.78 to 47.71%) than differentiated ones at all temperatures above 15°C (Table 2, Fig. 1A). No snail ventilated its lung at 10°C and only 33% of them executed a few ventilations at 15°C. No effect of temperature but a significant one of sexual differentiation was detected on the number of pumping movements per lung ventilation (Fig. 1B); the general mean across all temperatures was significantly lower for undifferentiated snails than for males and females (back-transformed means: 7.65 vs. 9.60, respectively). The duration of each pumping movement decreased significantly with temperature; the interaction with sex or differentiation was significant though lacked a clear pattern. No difference between males, females and undifferentiated snails was detected (Fig. 1C). For all snails on average, the duration of each pumping movement decreased abruptly from 2.69 s at 15°C to 1.15 s at 20°C and then declined gradually to 0.71 s at 30°C.

As undifferentiated and differentiated snails differed not only in the degree of maturation but also in size, one-way ANCOVAs were performed to investigate whether an effect of size *per se* existed, using shell length as covariate (log-transformed). The group with the wider size range (sexually differentiated snails) and the temperature range where aerial respiration was more frequent (25–30°C) were

TABLE 3. Summary of one-way ANCOVAs of lung ventilation frequency (VF), number of pumping movements per lung ventilation (NP) and duration of each pumping movement (DP) (square root-transformed) of differentiated *Pomacea canaliculata* snails with shell length (L) as covariate (log-transformed); only the data for 25ºC and 30ºC were included in the analyses.

Variables	Slope equality	Common slope	Slope nullity	Intercept equality	Back-transformed adjusted means
VF	$F_{1,42} = 0.05$ $P = 0.8168$	4.6389	$F_{1,43} = 28.57$ $P < 0.0001$	$F_{1,43} = 19.89$ $P = 0.0001$	25ºC: 2.92 30ºC: 6.79
NP	$F_{1,39} = 0.53$ $P = 0.4704$	1.2687	$F_{1,40} = 7.99$ $P = 0.0073$	$F_{1,40} = 0.10$ $P = 0.7534$	25ºC: 9.54 30ºC: 9.74
DP	$F_{1,39} = 3.69$ $P = 0.0618$	-0.0869	$F_{1,40} = 2.35$ $P = 0.1330$	$F_{1,40} = 82.30$ $P < 0.0001$	25ºC: 0.96 30ºC: 0.84

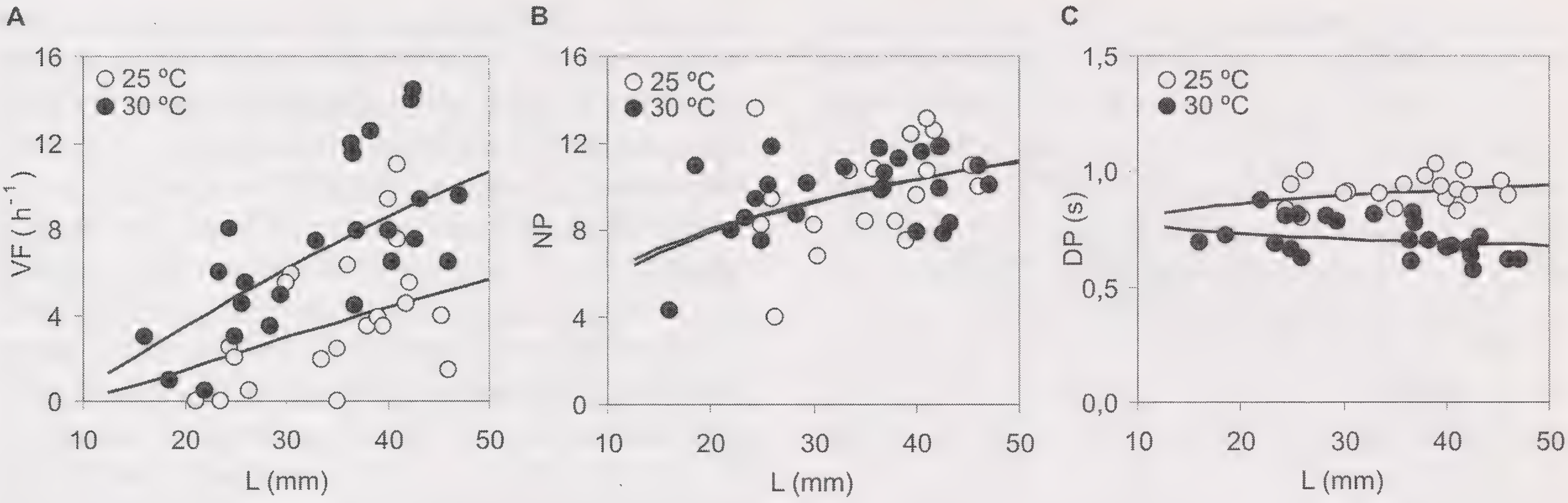

FIG. 2. Scatter plots of aerial respiration variables of differentiated *Pomacea canaliculata* snails vs. total shell length (L) at 25ºC and 30ºC: A) lung ventilation frequency (VF), B) number of pumping movements per lung ventilation (NP) and C) duration of each pumping movement (DP) (prediction curves of the aerial respiration variables (square root-transformed) vs. shell length (log-transformed) were estimated by least squares for each temperature).

selected for these analyses. Lung ventilation frequency increased significantly with shell length, the rates of increase (slopes) were not different at 30 and 25°C; the adjusted means (intercepts) were higher at 30°C (Table 3, Fig. 2A). The number of pumping movements also increased with snail size but, as pointed out in the ANOVA, no effect of temperature was evident (Table 3, Fig. 2B). The duration of each pumping movement was not affected by snail size (Table 3, Fig. 2C).

The slopes of the relationships of average and maximum depths with shell length were significantly higher for undifferentiated snails than for females and males (Table 4, Fig. 3). Males had slightly lower adjusted means for both average and maximum depths; a higher variability for females was also apparent. The great dispersion of depths was mainly due to the different degree of extension, curvature, and direction of the siphon relative to shell position. In all events of aerial respiration observed in the two series of trials, the snails were adhered to the lateral walls of aquaria during the entire process of lung ventilation. No snails were observed spontaneously releasing their foothold to float to the surface.

Ninety-four percent of snails observed within three hours of hatching (n = 872) contained an air bubble in the lung. Lung ventilation consisted of the short extension of the left nuchal lobe, without forming a closed tube or siphon but folding it to form a furrow dorsally closed by the mantle edge; the air bubble was captured

TABLE 4. Summary of one-way ANCOVAs of average (AD) and maximum depth (MD) during lung ventilation of *Pomacea canaliculata* with shell length (L) as covariate (log-log-transformed) (slope equality was first checked for females, males and undifferentiated (F, M, U) snails, and then the analysis was continued with the groups with non-significantly different slopes (F, M)).

Variables	Slope equality (F, M, U)	Slopes	Slope equality (F, M)	Common slope	Slope nullity	Intercept equality	Adjusted means*
AD	$F_{2,208} = 12.44$	F: 0.443^a M: 0.967^a	$F_{1,167} = 1.45$	0.7477	$F_{1,168} = 12.05$	$F_{1,168} = 5.52$	F: 15.038
	$P < 0.001$	U: 4.489^b	$P = 0.2309$		$P < 0.001$	$P < 0.02$	M: 12.363
MD	$F_{2,208} = 10.16$	F: 0.781^a M: 0.879^a	$F_{1,167} = 0.07$	0.8383	$F_{1,168} = 21.42$	$F_{1,168} = 6.29$	F: 23.408
	$P < 0.001$	U: 3.499^b	$P = 0.7887$		$P < 0.001$	$P < 0.02$	M: 19.635

[a,b] Different letters indicate significantly different slopes with Student t test (p < 0.001 after Dunn-Sidák correction)
*Back-transformed logarithmic adjusted means (mm)

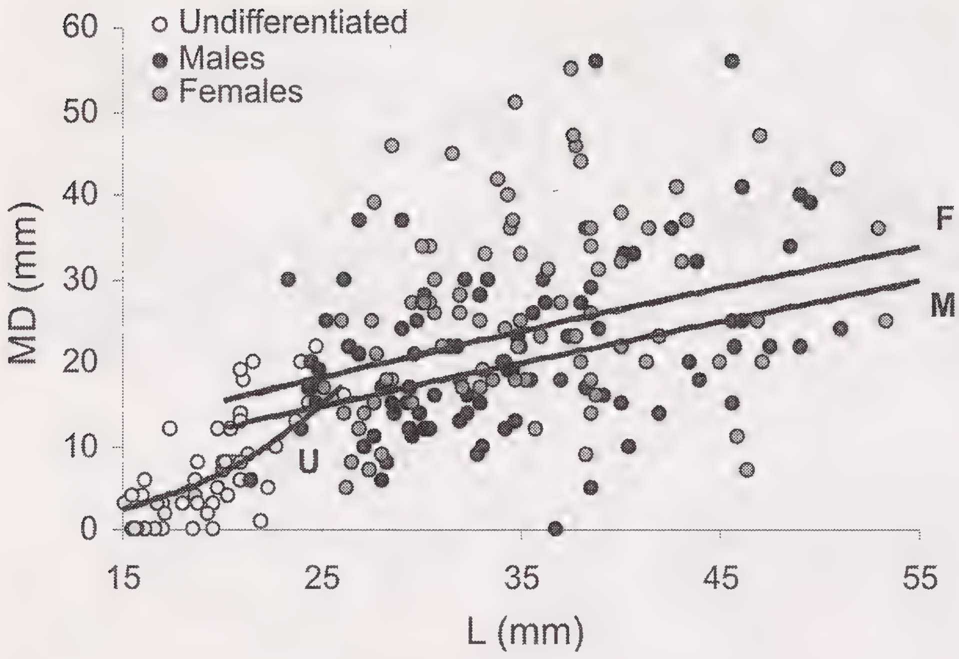

FIG. 3. Scatter plot of maximum depth during lung ventilation (MD) of *Pomacea canaliculata* vs. total shell length (L) (prediction curves were estimated by least squares after log-log transformation for female (F), male (M) and undifferentiated snails (U)).

in a single step, without any apparent pumping movement. The hatchlings usually take air while gliding on the water surface layer or when they first contact the surface after falling from the egg mass. In 95% of the cases, the snails removed from the calcareous shell of eggs in the last stage of development (n = 114) lacked an air bubble in the lung.

DISCUSSION

In *Pomacea canaliculata* aerial respiration begins within three hours of hatching (at 20–25°C) and is performed routinely as long as the snails are active; the lack of ventilation activity at 10°C was related to an almost total immobility of the snails since 94.5% remained fully retracted though not buried in the sand layer. In the southern Pampas, *P. canaliculata* remains in a lethargic state under rocks or among submersed plants for several months (Estebenet & Martín, 2002) although burying in mud to overwinter has also been suggested (Bachmann, 1960; Damborenea, 1996). *Pomacea paludosa* (Say 1829) fully retracts below 8°C, but remains immobile with the operculum slightly protruded beyond the shell aperture between 9°C and 13°C, probably to allow gill ventilation (Stevens et al., 2002). In the Curamalal stream even during the coldest months, part of the snails are found in the same state (unpublished data), indicating that eventually they open the operculum to allow some water circulation and they do not bury themselves; however they may end up buried as a result of siltation. Although 94.5% of the snails were not retracted at 15°C, only a few ventilated the lung during the two hour observation period, indicating that the interval between ventilations is greater than two hours.

The range of temperatures used here (10–30°C) is narrower than the annual fluctuations recorded in Curamalal stream (0.3–31.5°C) but includes the whole range in which the snails are active in that water-body. According to water temperature records the ventilatory activity of *P. canaliculata* in Curamalal stream would be almost null between April and September, and hence its microdistribution would be independent of the accessibility to water surface. On the other hand, during Spring and Summer (between October and March) the higher temperatures would induce an increase in aerial respiration that could restrict snails' microdistribution due to the need of periodical access to air. Darby et al. (2002) showed that *Pomacea paludosa* avoids depths greater than 50 cm and suggested that it could be due to a combination of a difficulty in reaching the water surface to breathe and unsuitable substrate or food.

For an "amphibious" snail the effect of temperature on aerial respiration may be due to a combination of a decline in gill oxygen intake due to a decrease in dissolved oxygen and an increase in metabolic rate. In *P. canaliculata,* lung ventilation frequency increases 6.76-fold and the duration of each pumping movement decreases 0.63-fold between 20 and 30°C, resulting in a total 4.24-fold increase in the time devoted to lung ventilation. Although *Pomacea* spp. are able to regulate oxygen intake with decreasing dissolved oxygen (metabolic O_2-regulators; Santos et al., 1987; Hanley & Ultsch, 1999), the small drop in that temperature range (17.6%; Margalef, 1983) seems not to be the main component of the increase in lung ventilation. On the other hand, the increase in specific oxygen consumption between 20 and 30°C for other *Pomacea* spp. (2.08 to 2.19-fold; Freiburg & Hazelwood, 1977; Santos et al., 1987) is also lower than the increase in the time devoted to lung ventilation in *P. canaliculata*. However, these rates belong to snails confined to small flasks and deprived of food so the response of activities that could contribute to raise metabolic rate (i.e., crawling and feeding) is very limited.

McClary (1964) registered for *P. paludosa* a 2.7-fold decrease and a 1.42 increase in the ventilation frequency for rapid changes (five minutes) in water temperature from 26 to 20°C and 26 to 30°C, respectively. For *P.*

canaliculata we estimated (for sexually differentiated snails after 72 h of acclimation) a 3.19-fold decrease for the range 25 to 20°C and 1.81-fold increase for 25 to 30°C. Perhaps the lower values in the former case indicate that the snails' bodies had not time enough to reach the water temperature during the one-hour period of observation. McClary (1964) also reported that lung ventilation increases under subsaturation levels of dissolved oxygen in *P. paludosa*; therefore, our observations of lung ventilation frequency should be considered as minima since they were made under conditions where aerial respiration is less promoted (i.e., dissolved oxygen near saturation).

Lung ventilation frequency showed a positive relationship with snail size (e.g.: 0.70 h^{-1} to 4.35 h^{-1} for 15 to 40 mm snails at 25°C, respectively). Assuming an isometric or simple scaling growth, the relative increase in ventilation frequency with snail size may be due to a decrease in the gas exchange surface relative to body mass (both in the gill and cutaneous surface). In fact, body mass grows even faster relative to shell length than expected by simple scaling (15.2%; Estebenet, 1998), probably indicating an increase in the relative fullness of the shell with flesh, since the overall shell shape changes little during growth (Estebenet et al., 2006). Contrarily to the relationship reported here, in the tropical apple snail *Pomacea urceus* (Müller 1774) lung ventilation frequency decreases with body size from 3.02 h^{-1} for juvenile snails (22–32 mm) to 0.96 h^{-1} for adult snails (90–125 mm) at 29°C (Burky & Burky, 1977). This dissimilar behaviour of ventilation frequency relative to size may be related to the fact that adult *P. urceus* aestivate buried in the mud during the dry season, displaying a set of reproductive, behavioural and physiological adaptations that includes the depression of the metabolic rate to one fifth of that of active snails (Burky et al., 1972).

The duration of each pumping movement decreased asymptotically with temperature, suggesting that the velocity of muscular contraction is the limiting factor in the response of this variable. The duration of each pumping movement at 25°C was of 0.89 s which compares well to the value of 0.9 s at 26°C registered in *P. paludosa* (McClary, 1964).

The number of pumping movements per ventilation was the only variable that showed no response to temperature but increased with size within sexually differentiated snails; size probably also explains the greater number of pumping movements of differentiated relative to undifferentiated snails. The values for this variable ranged from 7.06 for 15 mm snails to 10.59 for 45 mm snails. Similarly, in *Pomacea urceus* the number of movements at 29°C ranged between 8 and 18 for snails of 22–32 mm and 90–125 mm, respectively (Burky & Burky, 1977); the number of pumping movements at 26°C for immature and mature individuals of *P. paludosa* were 15 to 18.7, respectively (McClary, 1964). The increase with size is probably related to an increase in the volume of the respiratory dead space, that is, the volume of air in the lung or the siphon not in direct contact with the gas exchange surface. Since the lung is a simple sac without internal folds (Andrews, 1965) and siphon length increases with snail size, a higher number of pumping movements is required to replace the air in the lung in large snails. In fact, a simple opening of the pneumostome without any pumping is apparently enough in the hatchlings of *P. canaliculata*.

The number of pumping movements observed here is close to those recorded in most species of similar adult size – 10.6 and 9.8 for *Pomacea falconensis* (Pain & Arias, 1958) and *Pomacea luteostoma* (Swainson, 1832), respectively (Burky & Burky, 1977). Although the values of 18.7 reported for adults of *P. paludosa* (McClary, 1964) are clearly higher, this may be related to differences in the relative siphon length or in lung volume.

Despite the noteworthy sexual dimorphism of *P. canaliculata* in several ecological and morphological traits (Estebenet & Martín, 2002; Vega et al., 2005; Estebenet et al., 2006), the aerial respiration variables investigated here showed no consistent differences between sexes. Likewise, oxygen consumption rates are similar for males and females in *P. paludosa* (Freiburg & Hazelwood, 1977) and *P. lineata* (Santos & Mendes, 1981). On the other hand, females ventilate the lung at greater depths than males, though this slight difference seems of little ecological significance. Perhaps this is a correlate of subtle differences in shape, curvature or length of the siphon. In females, the massive albumen gland bulges from the floor of the pallial cavity, occupying most of it (Andrews, 1965) and probably distorts the position of respiratory organs.

Two main roles have been assigned to the siphon in apple snails: to diminish their visibility to aerial predators during lung ventilation and to reach the water surface through dense mats of vegetation (Freiburg & Hazelwood, 1977). Paradoxically, *Pomacea* spp. have the longest siphon among neotropical genera of apple snails (Andrews, 1965) and are the preferred prey of the snail kite *Rostrhamus sociabilis* (Snyder & Kale, 1983; Bennetts et al., 1994). Although the precise way in which the apple snails are detected is unknown, the extended siphon is probably the most visible part of the snail (Stevens et al., 2002), since shells are cryptic due to their colour, banding pattern and the presence of epibiontic algae. Nevertheless, since floating *P. canaliculata* snails are very rarely observed both in the laboratory and in the field, the success of a capture attempt depends on the ability to accurately locate and grab the shell beneath water surface. Although the entire range of depths recorded here (up to 6 cm) is well within the maximum potential reach of the snail kite (up to 16 cm; Sykes, 1987), the variability in siphon extension, curvature and direction could be critical to reduce the chance of an accurate localization in turbid water-bodies, which are the most frequently inhabited by *P. canaliculata* in the southern Pampas (Martín et al., 2001).

Independent of sexual differentiation, the depth during lung ventilation in *P. canaliculata* increases markedly beyond a shell length of approximately 20 mm. This would imply a heavier predation risk on small snails since they breathe air almost at the surface. However, *Pomacea* spp. smaller than 25 mm are rarely captured (e.g., Collett, 1977; Sykes, 1987; Tanaka et al., 2006), probably due to the combined effects of their low surfacing frequency (Bourne, 1993; our study), their low profitability (Beissinger et al., 1994), or a mechanical incapability of the snail kite talons to grasp snails below a certain diameter (Collett, 1977). The rapid increase in depth during lung ventilation could be interpreted as a behavioural response to counteract the raise in predation risk as snails grow beyond 20 mm.

Apple snails evolved from an aquatic ancestor with a ctenidium and developed a secondary lung inside the mantle cavity associated with an enlargement of shell aperture (Berthold, 1989). The other group of "amphibious" freshwater snails, the tiny pulmonate Basommatophora arose from a terrestrial lineage with a mantle cavity transformed into a lung and without a ctenidium (McMahon, 1983). According to Schmidt-Nielsen (1997), the interchange of air between the lung and the atmosphere occurs by ventilation only in vertebrates, though those of the siphonate apple snails (e.g., *Pomacea*, *Pila*, and *Marisa*) are clearly ventilation lungs (Andrews, 1965; Burky & Burky, 1977; Freiburg & Hazelwood, 1977; Berthold, 1991; our study). In such small invertebrates as pulmonate snails, the interchange occurs by diffusion through the pneumostome (McMahon, 1983; Schmidt-Nielsen, 1997), as seems to happen in the hatchlings and small juveniles of *P. canaliculata* (our study) and also in adult apple snails without the left nuchal lobe developed into a siphon (e.g., *Saulea*, *Lanistes*; Berthold, 1991). Therefore, besides the ontogenetic effect of snail size on the number of pumping movements, the occurrence of ventilation in snails with a lung seems to be related to the presence of a siphon and hence to the need of renewing the air from the increased respiratory dead space.

Our study showed that even when food is available only at the bottom and dissolved oxygen is near saturation, *P. canaliculata* snails routinely move to the water surface to ventilate the lung, despite of the reduction in the time and energy allocated to other activities and the potential increase in predation risk. This continuous crawling from the bottom to the surface seems to be a common feature of the behaviour of *Pomacea* spp. (McClary, 1964; Burky & Burky, 1977; Freiburg & Hazelwood, 1977), especially in habitats with warm and poorly oxygenated waters. Furthermore, McClary (1964) reported that *P. paludosa* snails, even under dissolved oxygen saturation, tend to crowd under barriers that impede their access to the surface. The basis of the need for a periodical ventilation of the lung and the acute and chronic consequences of a prolonged lack of access to air in *P. canaliculata* are both unknown. The mechanism and degree of air dependence need to be clarified to reach a better comprehension of the effects of lung ventilation on distribution at different spatial scales and also on the time allocation relative to other routine activities.

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POPULATION DYNAMICS OF AN URBAN POPULATION OF THE LAND SNAIL *MARMORANA SERPENTINA* (GASTROPODA: PULMONATA)

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INTRODUCTION

Spatial distribution, population structure, and dispersal patterns are fundamental characteristics of species. When the area occupied by a population far exceeds the dispersal ability of individuals, random mating cannot be assumed over the entire population. Wright (1946) introduced the concept of "neighbourhood" for cases in which individuals have only a limited number of potential partners, their neighbours. Neighbourhoods are restricted areas where random mating is likely. Study of the population structure and understanding of speciation processes depend on accurate data on movement patterns, dispersal capacity, and density (Parmakelis & Mylonas, 2004).

Land snails are particularly subject to population subdivision and geographic structuring due to their low dispersal capacity and frequent restriction to calcareous substrata. Active dispersal of terrestrial gastropods is influenced by such factors as population density, habitat complexity, climatic conditions, body size, and homing tendency (Cowie, 1980; Baker, 1988; A. Baur & B. Baur, 1990; Aubry, 2006).

This paper reports a study of population structure and monthly and daily activity of a non-native population of the land-snail species *Marmorana serpentina* (Férussac, 1821) living in the walls of the fortress of Siena, Italy. This species occurs in Sardinia and Corsica, but a few introduced populations are also present in Tuscany originating from passive transport by humans. *Marmorana serpentina* is a medium-size snail (1.5–2.0 cm shell diameter). It has a life cycle of at least three years. It inhabits rocky, preferably calcareous substrata. Mating occurs in spring and autumn with a period of aestivation between. Eggs are usually deposited in rock crevices and hatch after a few weeks.

MATERIALS AND METHODS

Survey Site

The study lasted from March 2006 to November 2007 and was performed at the Fortezza Medicea in Siena, a military fortress built in the sixteenth century. The fortress walls offer advantages over natural habitats. Surveys can be more exhaustive and complete. Density estimates are more accurate because of the simplified habitat surface and the high probability that a marked specimen not recaptured left the surveyed plots or died. Survey can be frequent, which is particularly important for monitoring daily activity. After a preliminary survey, the side of the fortress with the highest population density was chosen. It faces ESE and is 70 m long and 6 m high (Fig. 1).

The fortress walls were built with clay bricks cemented with lime mortar. Brick surfaces are separated by columns of sandstone every ten meters. Crevices between the bricks, homogeneous in shape, size, and distribution, provide shelter for snails.

Insolation of the wall varies during the day: for the first half of the morning sunlight illuminates only the southern half of the wall, which has a patchy cover of algae, lichens, and occasional grasses. Different lichen associations are evident on the wall: on the northern half there are only crustose lichens, such as *Physcia adscendens* and *Xanthoria parietina*; on the southern half, there are crustose lichens with a particularly developed thallus, such as *Physconia grisea*. Algal cover is abundant, especially on the northern half. More than 5 m above the ground, vegetation becomes sporadic probably due to renovation.

Marmorana serpentina occupies the first 5 m from the ground. Above this height no land snails seem to be present.

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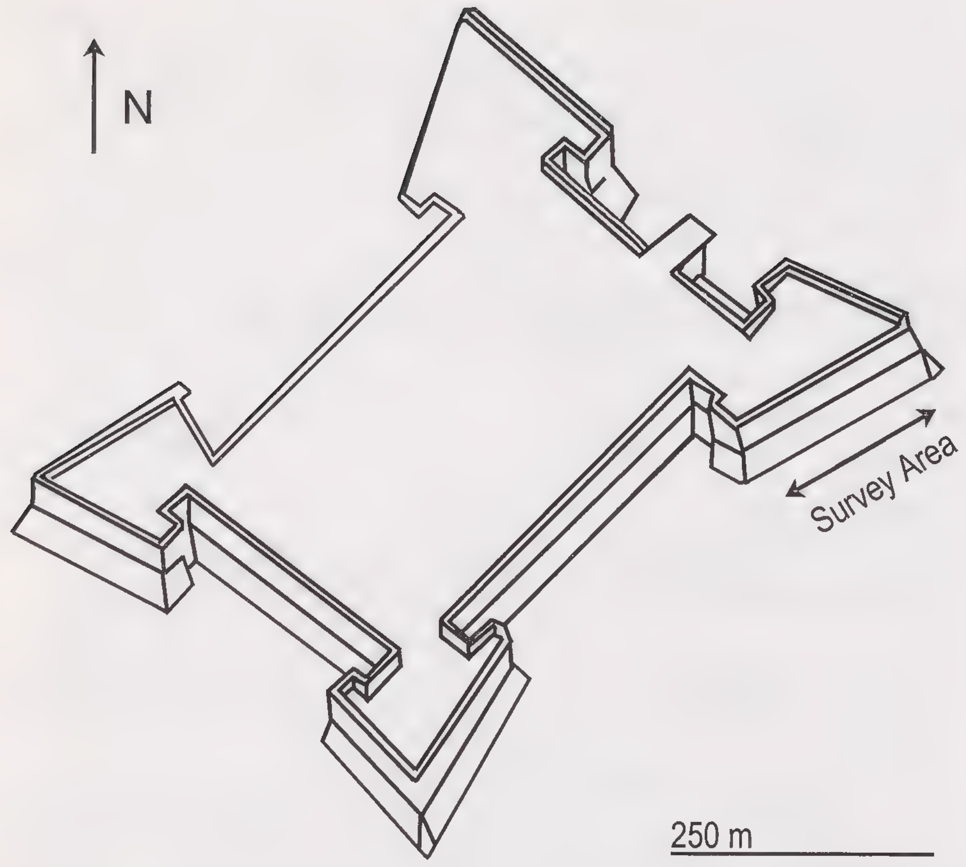

FIG. 1. The sampling site: the Renaissance Fortezza Medicea in Siena.

Data Collection

The study area was stratified into seven contiguous strips measuring 10.0 m by 2.5 m (Fig. 2). This stratification was simple because the wall is divided by columns of sandstone every 10 metres. Density was assessed by randomly placing five 1 x 1 m sampling squares per strip. Surveys were undertaken in 2006 and 2007 at intervals of at least 15 days (Table 1).

After field assessment, permanent marker was chosen as the most suitable marking method (Parmakelis & Mylonas, 2004). We searched for adult (identified by shell size and fully developed shell lip) and juvenile snails in each strip and marked them with a numerical code, when possible without detaching them from the wall or disturbing them excessively. In 2006, 106 specimens were marked on the first survey occasion (March); in 2007, 125 specimens were marked at the start (March).

Each strip was imagined as a bivariate Cartesian plan and used to record the position of marked specimens. In order to record long distance dispersal, we also searched outside the surveyed area (10 m on either side). During each survey the distance between consecutive positions of recaptured specimens was calculated – Euclidean distance:

$$d = \sqrt{(X_1 - X_2)^2 + (Y_1 - Y_2)^2}.$$

From summer 2007, daily activity and environmental variables were recorded (Table 1).

TABLE 1. Survey plan. M, marking; N, number of marked specimens; D, density; R, recapture; DA, daily activity; EV, environmental variables recorded.

N°	Date	Recorded variables
1	03-03-2006	M 1 (N = 106); D
2	19-03-2006	R; D
3	25-04-2006	R; D
4	30-05-2006	R; D
5	28-06-2006	R; D
6	01-03-2007	M 2 (N = 125); D
7	22-03-2007	R; D
8	30-04-2007	R; D
9	17-05-2007	R; D
10	31-05-2007	R; D
11	14-06-2007	R; D
12	28-06-2007	R; DA; EV; D
13	12-07-2007	R; DA; EV; D
14	03-09-2007	R; DA; EV; D
15	13-09-2007	R; DA; EV; D
16	28-09-2007	R; DA; EV; D
17	11-10-2007	R; DA; EV; D
18	26-10-2007	R; DA; EV; D
19	12-11-2007	R; DA; EV; D

During the first two daily activity surveys, the positions of recaptured specimens were taken six times (every three hours from 9 am to 12 pm), but since this timing was too intense for the low activity of the snails, from the third survey it was subsequently only recorded four times (9 am, 3 pm, 6 pm, and 12 pm). Temperature (T) and relative humidity (RH) were recorded with a Comet data logger (model S3120). Resolution and accuracy were 0.1 and ± 2.5% and 0.1 and ± 0.4°C, respectively. Three randomly located replicates of RH and T were recorded at each strip for each survey and each 1 hr (on average) time period (9.00, 15.00, 24.00). Within each survey, T and RH were recorded at the beginning and end of each time period.

Statistical Analysis

Factorial analysis of variance was used to detect differences in dependent variables (density, Euclidean distance, temperature, relative humidity) across sampling factors (strips, days, time periods). The specific factorial design depended on the variables analysed. For density and Euclidean distance, strips and days were used as crossed factors, while for days on which daily activity was studied, we analysed the dependence of Euclidean distance on strips, days, and time periods. For T and RH, a four-factor design with strips, days, time periods, and minutes (the beginning and the end of time periods) was used.

Rayleigh's test (Zar, 1984) was used to check for directional preference in individual movements: the azimuth was defined according to the ordinate axis. Many indices can be used to define population aggregation. On the basis of the survey design, we chose the mean-to-variance ratio to test the null hypothesis of regular distribution (Krebs, 1998).

The specimens recaptured at least twice in the 2007 survey were classified on the basis of the point most distant from where they were marked: (1) not vagile: within 1 m; (2) slightly vagile: beyond 1 m but within 3 m; (3) vagile: beyond 3 m. Specimens were classified as fugitive if they were no longer found and as pulsing if they were found intermittently.

Finally, in order to estimate neighbourhood size (N_S), we used the formula introduced by Wright (1946) for a continuously distributed population of randomly mating simultaneous hermaphrodites distributed in a two-dimensional habitat: $N_S = 4\pi S^2 D$, where N_S is the number of individuals in the neighbourhood area, S^2 is the variance of dispersal of adults during one generation along a single axis, and *D* is the density of breeding individuals. Following Greenwood (1976), the variance of dispersal along a single axis was calculated as half the variance of dispersal in all directions. Because previous field observations (confirmed by the results of the present study) suggested that individuals may live at least three years and probably reach maturity in the first two years, we estimated a minimum generation time of 1.5–2 years and calculated S^2 accordingly. The associated area (N_A) was calculated as a circle of radius $\sqrt{2S}$ according to Pfenninger et al. (1996).

All statistical analyses were performed with the R package (http://www.r-project.org) and STATISTICA 5 (StatSoft Inc., Tulsa, USA).

RESULTS

Population Density and Environmental Features

Population density differed significantly among strips ($F_{6,\ 532} = 33.2$, $P < 0.001$) and days ($F_{18,\ 532} = 2.1$, $P < 0.001$), but the interaction between these two factors was not signifi-

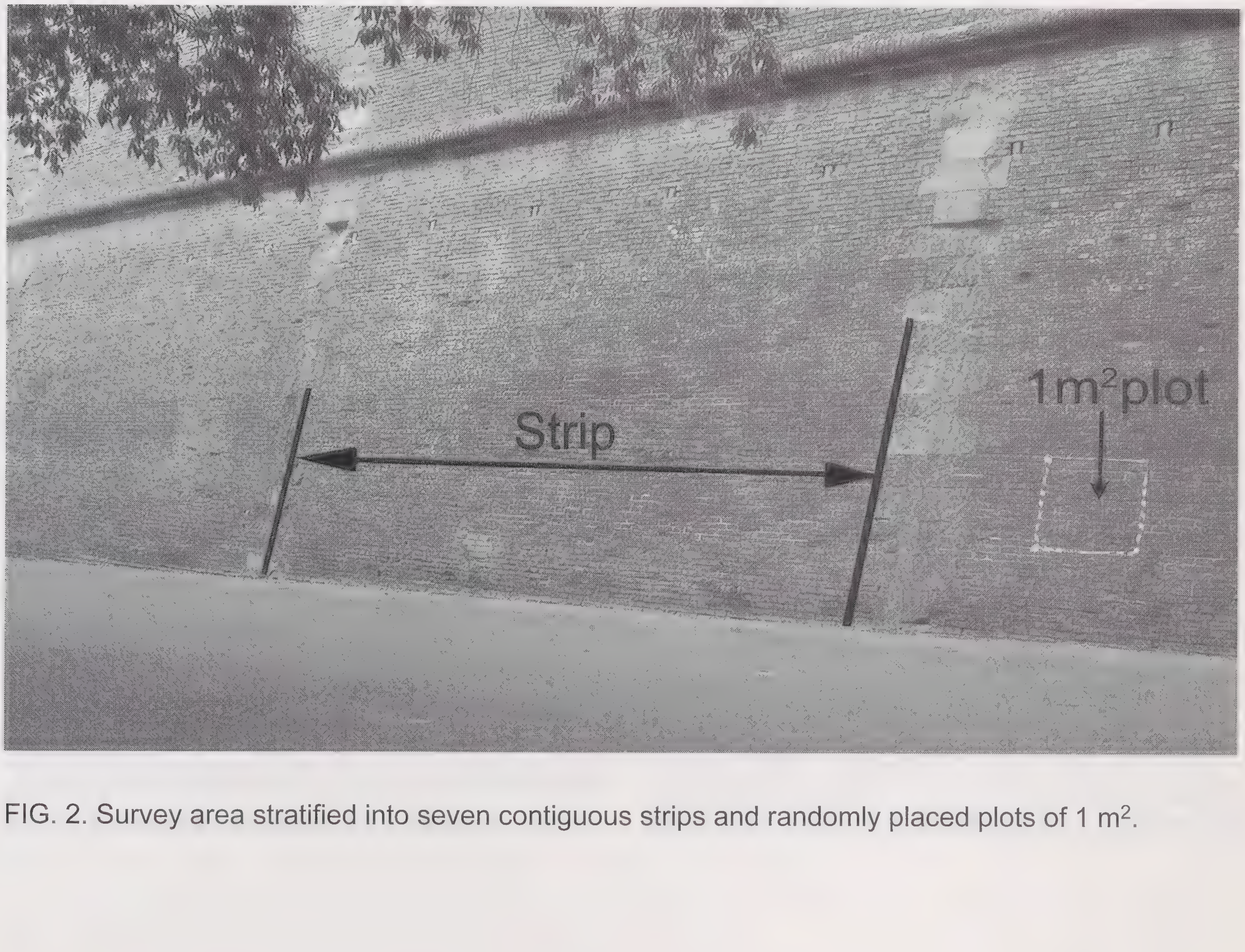

FIG. 2. Survey area stratified into seven contiguous strips and randomly placed plots of 1 m².

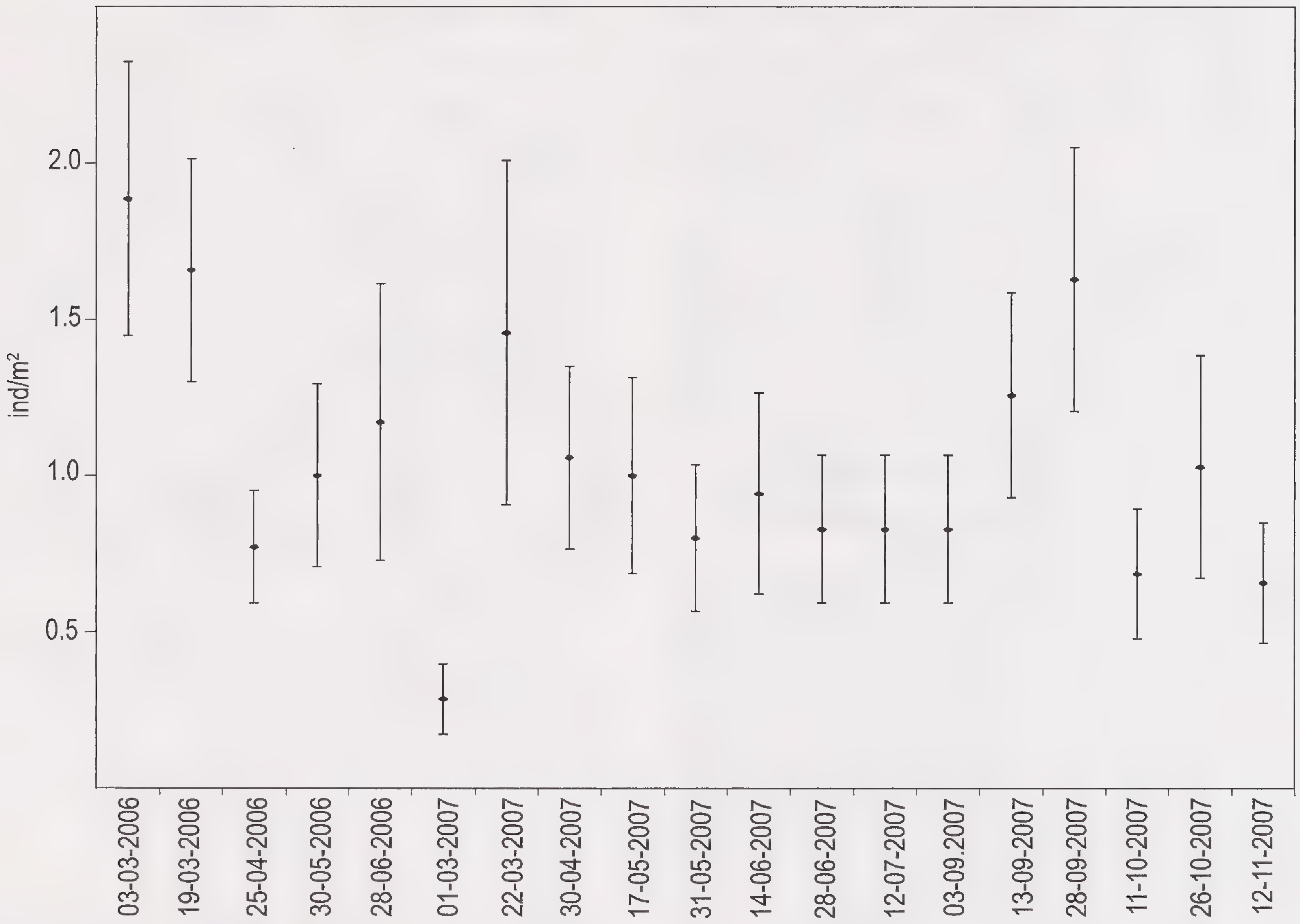

FIG. 3. Mean (± S.E.) population densities across surveys ordered chronologically from March 2006 to November 2007.

cant, indicating consistent spatial differences in time. Mean (± S.E.) density ranged from 0.2 ± 0.1 ind/m^2 (strip 6) to 2.3 ± 0.3 ind/m^2 (strip 3) and 2.4 ± 0.3 ind/m^2 (strip 2). Density decreased in the first year, culminating with the lowest observed density (0.3 ± 0.1 ind/m^2) in March 2007, after which the population recovered and oscillated around the 2006 mean of approximately 0.4 ind/m^2 (Fig. 3).

The variance-to-mean ratio test was calculated by pooling data of all days within each strip (N = 95). The aggregation index indicated that populations clustered significantly at the scale of the strips (Table 2).

The ANOVA model identified days as the main factor driving T ($F_{7, 998} = 643$, $P < 0.001$) and % RH ($F_{7, 990} = 490$, $P < 0.001$), while no significant spatial differences were detected among strips. The highest mean daily relative humidity was observed in autumn (67% RH at 18.5°C on 26 September 2007), the lowest in summer (28% RH at 25.5°C on 12 July and 13 September 2007).

Dispersal and Neighbourhood Size

Over the whole survey period, a total of 432 movements were recorded. The mean (± S.D.) percentage of recapture (Fig. 4) was 22 ± 9% in 2006 and 22 ± 7% in 2007. In the two years, six marked shells were found dead, accounting for a small percentage (< 5%) of non-recaptures.

No significant spatial variation and no differences between adults and juveniles were detected in mean Euclidean distance moved (dispersal). However, significant differences were found for adults among days ($F_{16, 318} = 5.9$, $P < 0.001$) with mean (± S.E.) distances ranging from 0.2 ± 0.1 m (28 June 2007) to 2.9 ± 0.6 m (12 November 2007) (Fig. 5). Among the eight surveys on which daily activity was

TABLE 2. Aggregation index (N = 95 for each strip).

Strip	Mean Density	Variance	χ^2 variance/mean
1	0.41	0.79	183***
2	2.39	9.73	383**
3	2.33	6.24	252**
4	0.63	0.85	127*
5	0.81	1.36	159**
6	0.17	0.22	127*
7	0.53	1.42	244**

***P < 0.001; **P < 0.01; *P < 0.05

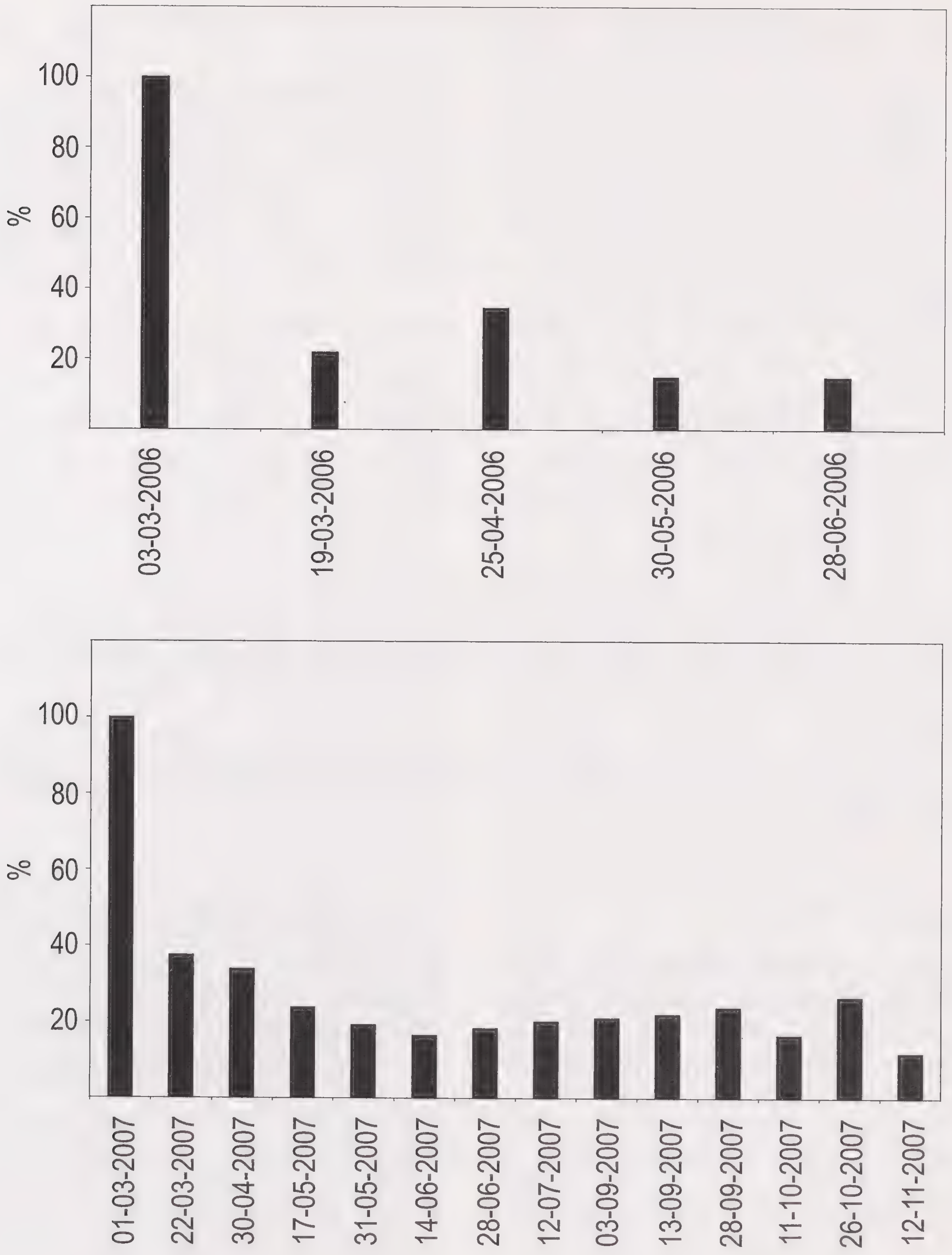

FIG. 4. Frequency distribution (percentage of total number of marked specimens) of recaptures after the initial marking date in March of each of 2006 and 2007.

studied, dispersal was only detected on rainy days. Mean (± S.E.) Euclidean distance of all measurements was 0.9 ± 0.1 m. However, observations on marked specimens showed different and apparently inconsistent movement patterns: some moved upwards or downwards and returned approximately to their original position, while others moved randomly around their starting point; some covered a total of about 3.5 m, but because they moved away and back to the starting point, the distance was only a few centimetres. Others covered a total of less than 0.5 m.

Rayleigh's test performed at several temporal scales (yearly, monthly, and daily) did not show any significant direction of dispersal, thus implying a random pattern. However, in a few cases (N = 7) specimens left the strips in which they were marked.

Chi square on the frequency distribution of the 69 specimens classified as fugitive or pulsing, to test the null hypothesis that the categories had the same frequency, demonstrated that fugitives were significantly less frequent ($\chi^2 = 32$, d.f. = 1, $P < 0.001$), whereas the frequency of the three vagility categories

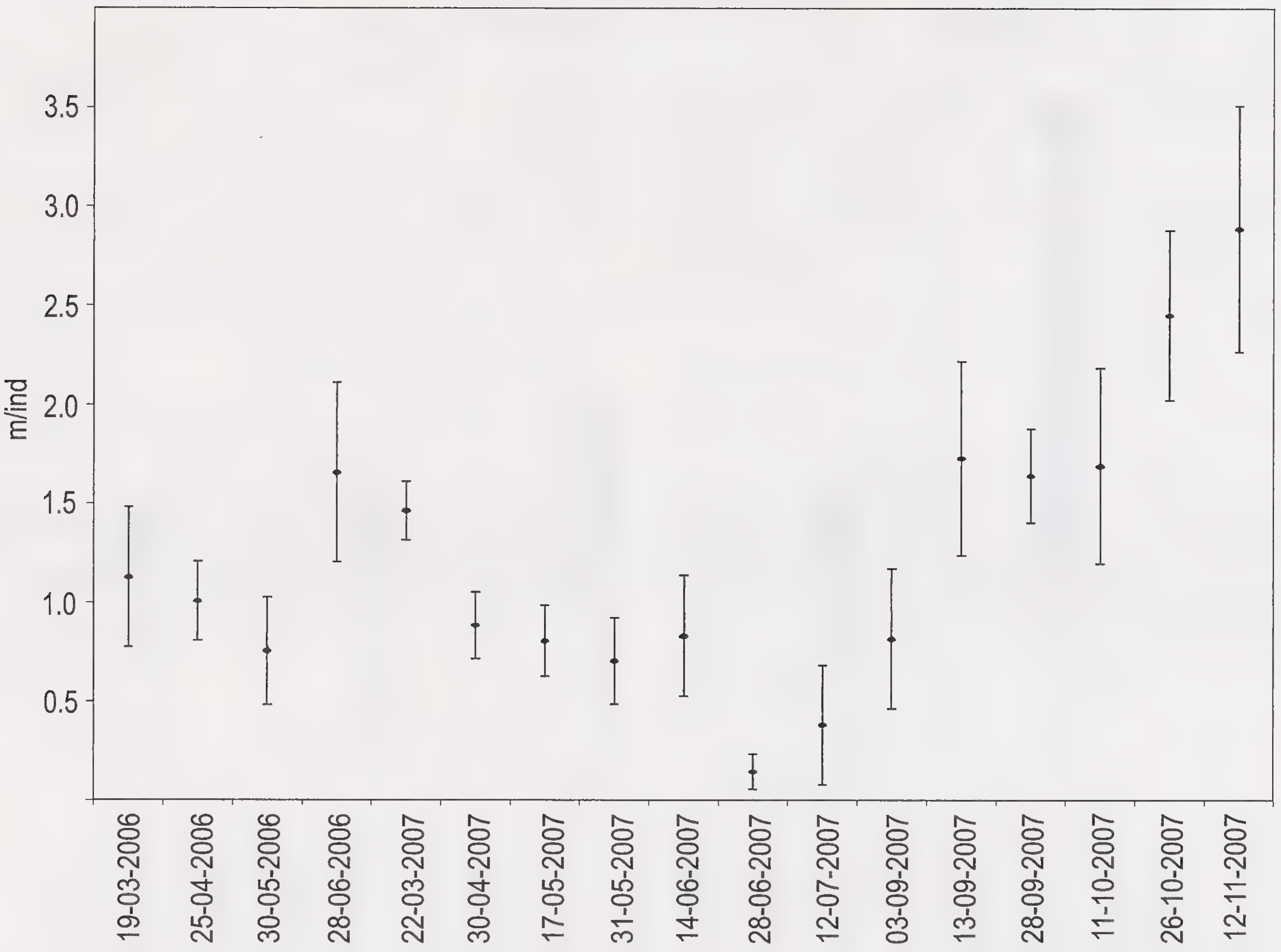

FIG. 5. Mean (± S.E.) Euclidean distance between two successive recaptures by date.

did not vary significantly ($\chi^2 = 0.72$, d.f. = 2, $P > 0.5$).

Since the strips supported different population densities and the differences remained temporally consistent, neighbourhood size and area were calculated for each strip, averaging the values obtained using the mean density observed in each strip at each date. Neighbourhood area was found to be 6.7 m^2 (radius = 1.5 m) and neighbourhood size *Ns* ($F_{6, 126} = 28.2$, $P < 0.001$) varied significantly from a mean (± S.E.) of 34 ± 4 in strip 2 to 2 ± 1 in strip 6 (Fig. 6).

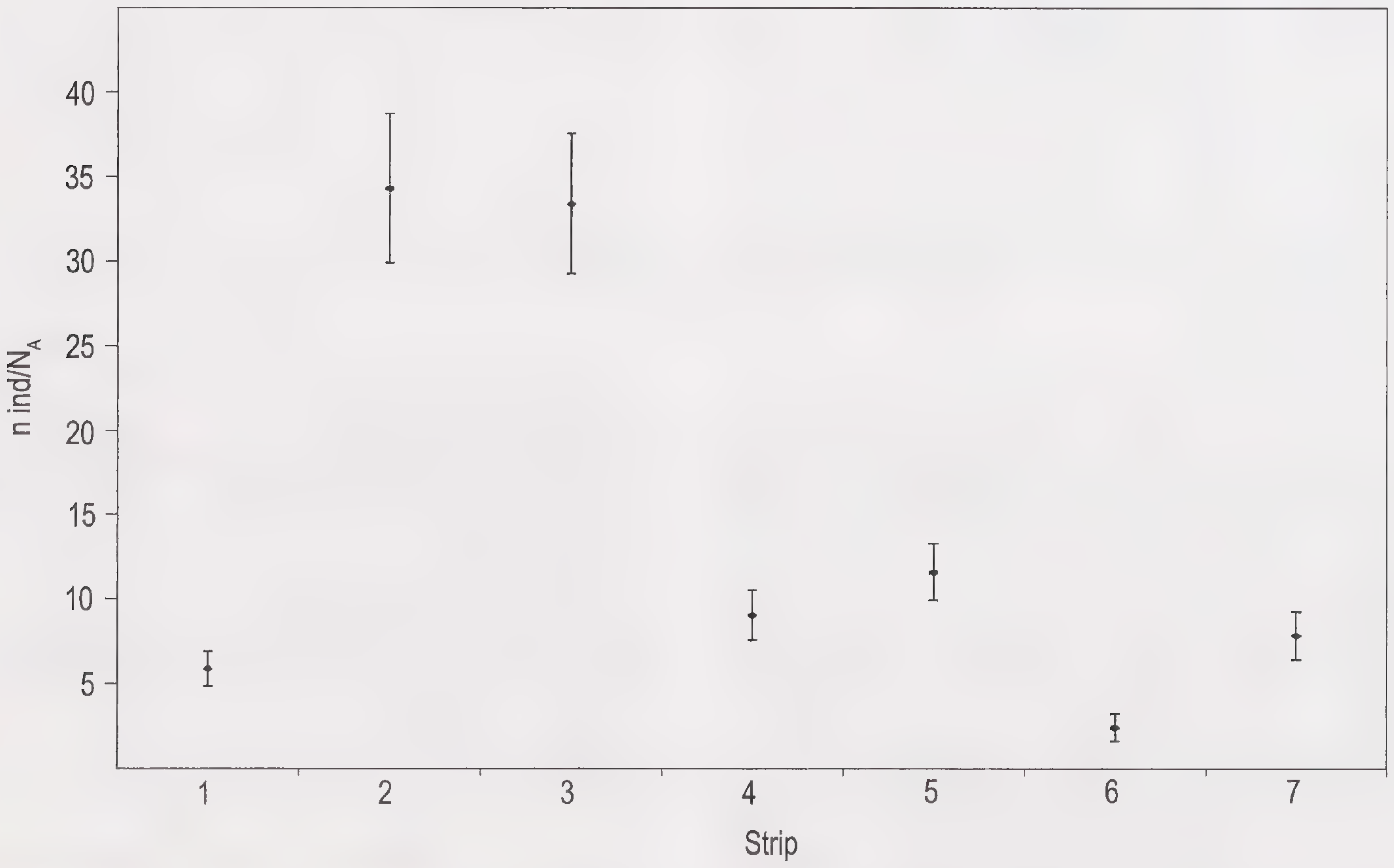

FIG. 6. Mean (± S.E.) neighbourhood size of the seven wall strips calculated over surveys. N_A: Neighbourhood Area.

DISCUSSION

The population densities recorded for *M. serpentina* were low, similar to those documented for two species of *Mastus* (Parmakelis & Mylonas, 2004), but one twentieth to one third those of other snails (Livshits, 1983; B. Baur & A. Baur, 1995; Pfenninger et al., 1996; Giokas & Mylonas, 2002).

Dispersal rate seems to be reduced (Greewood, 1974; Cameron & Carter, 1979) or increased (Cain & Currey, 1968; Oosterhoff, 1977) when densities are high. In the case of *M. serpentina*, no correlation between density and dispersal was found, perhaps reflecting the overall low density recorded. Significant differences in density on a scale of 10 m (i.e., between strips) were not correlated with environmental factors (T, % RH, and distribution of crevices, which was homogeneous, being spaces between bricks), but could possibly be correlated with spatial variation, which we did not quantify, of lichen and algal communities observed on the wall. In fact, insolation of the wall varies during the day and this may be the factor determining lichen and algal community structure (S. Munzi, comm. pers.), in addition to a possible but still not quantified effect of grazing invertebrates, including snails. Interestingly, the lowest density was recorded in the northern area (strip 6), where a crustose lichens with a particularly developed thallus (*Physconia grisea*) completely covers the wall, while only crustose lichens live on the rest of the wall. Rock-dwelling snails may have a limited ability to crawl on vegetation. Their sticky mucus enables them to adhere to vertical rock faces but makes moving on vegetation difficult (B. Baur & A. Baur, 1995). However, no data are available on the lichen preferences of *M. serpentina*. Further studies are required to test the hypothesis of significant relationships between the distributions of lichens, algae, and snails.

Recapture frequencies were in line with those in similar land snail studies (Pfenninger et al., 1996; Parmakelis & Mylonas, 2004). The decreasing trend in recapture frequencies (Fig. 4) did not seem due only to mortality, since no corresponding decrease in densities was observed. While this is not in itself proof, because increased mortality rate may be balanced by increased reproductive rate, overall observations did not support the hypothesis of higher reproductive rate. For instance, densities of juveniles remained stable over the two years. It is also unlikely that many of the non-recaptured specimens were missed: the relatively simplified environment of the wall made it easy to detect resting and hiding specimens. Specimens not recorded during certain surveys must have climbed beyond the upper limit of the survey areas. The sharp decrease in adult density from 2006 to 2007 may, on the other hand, have a demographic meaning.

Dispersal of *M. serpentina* was similar to that yearly recorded for other rock-dwelling snails: around 2.0 m for *Albinaria corrugata* (Schilthuizen & Lombaerts, 1994), 2.6 m on limestone pavement and 0.9 m on stone walls for *Chondrina clienta* (B. Baur & A. Baur, 1995).

Marmorana serpentina showed a dispersal ranging from 0.38 m to 2.90 m but with significant differences between surveys. High values coincided with the first rains after aestivation. In line with observations of other species (Yom-Tov, 1971; Cook, 2001), *M. serpentina* showed that a new epiphragm may be built after every bout of activity and is not associated with specific seasonal periods of enforced rest. In many terrestrial gastropods, breaking dormancy may not coincide with the beginning of activity after rest: individuals may become active after any minimum dormant period when conditions are favourable and may rest again immediately (Cook, 2001). It seems that hibernation and aestivation in shelled land snails have only a weak endogenous component, being largely triggered by prevailing weather conditions (Cook, 2001).

The high values of dispersal (Fig. 5) at the first rains after summer resting may be related to a demand for food and the distances travelled may increase with food deprivation (Airey, 1987; Rollo, 1991). During subsequent rainfall, snails mated, but the highest values of dispersal were lower (Fig. 5): when snails move to mate, they choose the closest partners, resulting in lower mean dispersal and the formation of groups contending for partners.

No significant direction of dispersal was detected, on spatial or temporal scales; evidence of random dispersal has been recorded for other land-snail species (A. Baur & B. Baur, 1993; Pfenninger et al., 1996; Parmakelis & Mylonas, 2004). Further, in *M. serpentina* and many other species, no evidence of homing (i.e. return to the same site by an individual) has been found, although clear fidelity to an area on a scale of 10 m x 5 m (i.e. one of the strips) was found. In two years of survey, more than half the marked specimens remained faithful to the area (circle of 1 to 3 m radius) in which they were marked (only seven specimens crossed the physical border separating wall strips). The absence of homing and directionality of dispersal, which could reasonably but mistakenly be postulated in an aggregated population structure, can be interpreted as selective responses to land life (Cook, 2001). In fact, random movements with low dispersal keep individuals faithful to an area of approximately 10 m x 5 m (i.e. one of the strips): individuals may search for resting sites, chosen simply by stopping at the first one encountered, which was previously occupied by a conspecific. Thus, homing may simply be viewed as returning to sites previously occupied by conspecifics (Cook, 2001), and in the case of the study population this is done by maintaining fidelity to a small area.

Neighbourhood size estimates for different land snails range from: 15 to 6,000 individuals (Greenwood, 1976; Crook, 1980; Cowie, 1984; Murray & Clarke, 1984; B. Baur, 1993; Schilthuizen & Lombaerts, 1994; Pfenninger et al., 1996; Parmakelis & Mylonas, 2004).

Neighbourhood size for *M. serpentina* was at the low end of this range. The study population showed significant differences in neighbourhood size at a very local scale (a 10 m scale between wall strips). Some studies (Schilthuizen & Lombaerts, 1994; Pfenninger et al., 1996; Parmakelis & Mylonas, 2004) have used an integrated approach to calculate gene flow in land snails by direct (mark-recapture experiment) and indirect (genetic variation) methods. No data on gene flow estimated by indirect methods is available for *M. serpentina*, but the patterns of population dynamics observed were similar to those of other species (low density, low dispersal and small neighbourhood size). The processes involved may be similar. Some studies indicate that when gene flow between demes is extremely limited, it can act as a homogenizing force only on a scale of a few tens of meters (Schilthuizen & Lombaerts, 1994). Beyond this scale, genetic drift and selection can play a key role in the genetic differentiation of demes (Schilthuizen & Lombaerts, 1994). Moreover, neighbourhood population structure, built by random dispersal processes in a limited spatial range, are probably a major force in generating and maintaining morphological and genetic variability (Pfenninger et al., 1996).

The present results show that population structure in *Marmorana serpentina* may be largely affected by limited and aggregated distribution due to their low dispersal capacity and fidelity to a small area. Although this conclusion stems from results gained on a non-native population, several widespread field observations on naturally occurring populations (Fiorentino et al., 2008a, b) indicate that densities can be locally lower and more aggregated than densities reported here, as well as similar to them.

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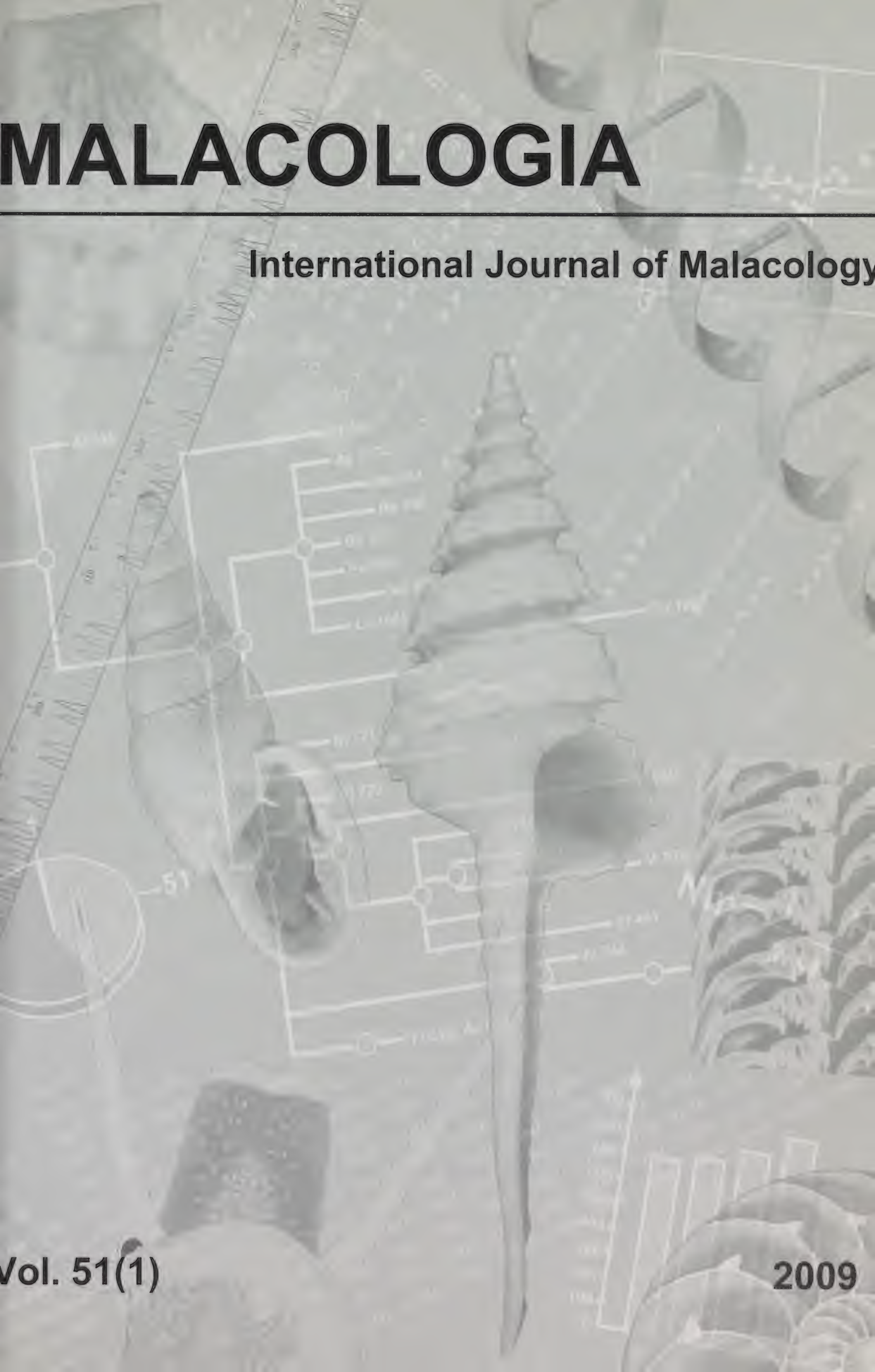
ERRATUM

Malacologia, 2008, 50(1–2): 293–302. K.-L. Kwong, P.-K. Wong, S. S. S. Lau & J.-W. Qiu: Determinants of the distribution of apple snails in Hong Kong two decades after their initial invasion.

It is regretted that the corresponding author's name was misprinted. The correct name is Jian-Wen Qiu.



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FEEDING RATES AND FOOD CONVERSION EFFICIENCIES IN THE APPLE SNAIL *POMACEA CANALICULATA* (CAENOGASTROPODA: AMPULLARIIDAE)

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ABSTRACT

Pomacea canaliculata is the only freshwater snail listed as one of the 100 worst invaders worldwide. Recent studies have demonstrated that small *Pomacea* snails have higher foraging and competitive abilities than larger snails and hence that ecological and agricultural damage of this invasive snail may be size-dependent. Furthermore, females of *P. canaliculata* usually reach larger sizes than males, a pattern that results from higher growth rates and not from higher survivorship in females; however, the proximal causes of the sexual dimorphic growth rates are unknown. In this study, we investigate the ingestion rates and growth efficiencies of *P. canaliculata* in order to explain the ontogenetic and sexual differences in growth and food consumption patterns. Two experiments were performed to study specific ingestion rates and the efficiency in food conversion to body mass at different feeding conditions. Ontogenetic and sexual differences were found in the specific ingestion rates. These decreased inversely with shell length and were higher for females than for males of comparable size. Conversion efficiencies decreased with age in both sexes, in males noticeably earlier than in females. Under high food availability conditions, the decrease is sharper than under low food availability. However, the effect of food availability almost disappeared when in the effect of size was removed. The sexual dimorphism of growth efficiencies and ingestion rates explain why females tend to reach a larger adult size than males, a pattern probably explained by development of the testicle and correlated reduction of mid-gut gland size. Our results on ontogenetic patterns of ingestion rates support predictions that during the reproductive season small snails may cause a great part of the damage to aquatic crops and natural wetlands.

Key words: growth, sexual dimorphism, allometry, invader, agricultural damage.

INTRODUCTION

Pomacea canaliculata (Lamarck, 1822) is native to South America and has been introduced in Asia, North America, and Hawaii for aquaculture and aquarium trade (Cowie et al., 2006; Rawlings et al., 2007; Hayes et al., 2008), where they have become established as serious pests of aquatic crops (Joshi & Sebastian, 2006). In addition, they are able produce major environmental changes resulting from the depletion of aquatic plants (Carlsson et al., 2004). *Pomacea canaliculata* has been listed as one of the 100 worst invaders worldwide and has the distinction of being the only freshwater snail on that list (Lowe et al., 2000). High growth and reproductive rates, polyphagous feeding habits, amphibious respiration, and aestivating capacity (Cowie, 2002; Estebenet & Martín, 2002) make *Pomacea* species highly adaptable and resistant to diverse environmental conditions, probably explaining in part both their pest status and the interest they attract as aquaculture organisms (e.g., Mendoza et al., 1999; Ramnarine, 2004).

Most research has focused recently on applied aspects of its biology and ecology (e.g., Tanaka et al., 1999; Carlsson et al., 2004; Yusa et al., 2006; Peña & Pocsidio, 2007), but some fundamental aspects of its basic biology remain obscure. Females of *P. canaliculata* reach larger sizes than males, both in natural habitats and when reared in the laboratory, a pattern that results from higher growth rates rather than from different survivorship rates (Martín & Estebenet, 2002; Estebenet et al., 2006). *Pomacea canaliculata* females always mature at an older age than males, independently of food availability (Estoy et al., 2002; Tamburi & Martín, 2009). In animals with asymptotic growth after maturity, the sex that matures later is usually the larger one (Stamps & Krishnan, 1997). However, the asynchronic maturity does not fully explain the sexual size dimorphism, because *P. canaliculata* are often dimorphic long before maturity (Estebenet & Martín, 2003). In spite of the generality of this pattern in this species, and its occurrence in other apple snails (Estebenet et al., 2006), the proximal causes of the dimorphic growth rates are still unknown. Higher ingestion rates or more efficient use of food by females appear to be the most plausible explanations.

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Higher ingestion rates for juvenile snails in relation to those of adults have been reported for *P. canaliculata* and *Pomacea insularum* (d'Orbigny, 1835) (Boland et al., 2008) and for the Indian apple snail *Pila globosa* (Swainson, 1822) (Hannifa, 1982). In field experimental enclosures with *P. canaliculata,* Carlsson & Brönmark (2006) demonstrated that small snails have higher foraging and competitive abilities than big snails, indicating that the degree of damage from this invasive snail to aquatic vegetation or crops may be size-dependent. Knowledge of size-dependent feeding rates may be useful for predicting damage to aquatic cultures and in developing food-efficient feeding regimes for apple snail aquaculture programs (Mendoza et al., 1999; Ramnarine, 2004).

In this study, we investigate the ingestion rates and growth efficiencies of *P. canaliculata* under different food availability conditions, with the goal of determining if these variables explain the ontogenetic and sexual differences in growth and resource consumption patterns reported for this apple snail.

MATERIALS AND METHODS

Source of Snails and General Rearing Conditions

The original source of all the snails used in this study was a population from the Curamalal stream (southern Buenos Aires Province, Argentina), which has been the subject of previous studies (Martín & Estebenet, 2002; Estebenet & Martín, 2003). All experiments were performed in a rearing chamber at 25 ± 3°C and a 14:10 h (light:dark) photoperiod. Snails were reared in tap water saturated with CO_3Ca and fed on fresh lettuce only.

Experiment 1: Ingestion Rates

The snails used to determine the daily ingestion rates were obtained from laboratory stocks (originated from field collected egg-masses) and supplemented with field-collected snails that had been maintained in laboratory conditions for at least a week. They were all fed *ad libitum* during three days before the begining of the trials. Snails were carefully observed to detect the testicle through the translucent shell (Takeda, 1999) and the humping of the operculum in males (Estebenet et al., 2006). These traits were clearly noticeable in males whose shell was longer than 15 mm, so that smaller snails were categorized as undifferentiated and bigger ones, lacking male traits, as females. The same method was used in Experiment 2, where all the snails were correctly sexed (confirmed by oviposition in females and by the copulation behavior in males; Tamburi & Martín, 2009).

To estimate food ingestion relative to size, snails with shell lengths from 5 to 55 mm were maintained with weighed surplus lettuce in a rearing chamber for 24 hours to account for daily rhythm in activity and feeding (Heiler et al., 2008). Fresh hydrated lettuce, drained by gentle centrifugation, was weighed with an analytical scale at the start and at the end of the experiment to quantify the amount consumed. Shell length (SL, mm), from the apex to the lower margin of the peristome (Estebenet & Martín, 2003), was measured for each snail with a caliper (for snails larger than 10 mm) or under a stereoscopic microscope with micrometric lens. With the aim that the total weight of lettuce consumed was in agreement with the precision of the scale (0.1 mg), the consumption of snails larger than 15 mm was estimated individually, while 19 sets of 2 to 15 individuals of similar size (maximum CV% for shell length = 6.3) were used for smaller ones. To avoid feeding interference among individuals, the volume of the aquaria and the amount of lettuce were well in excess relative to the whole mass of snails and their consumption. Live snail weight (LW, g) was obtained individually or for the set of snails of each feeding trial, respectively (after allowing the snails to crawl over a plastic surface for a few minutes to release the water retained in the pallial cavity). To account for hydration effects, the lettuce consumed was corrected by a factor obtained from the weight gain of similar amounts of lettuce in four control aquaria without snails.

The specific ingestion rate (SIR, d^{-1}) was calculated as the lettuce consumed (LC, g) per day relative to the snail live weight (LW, g). After a log-log transformation to linearize the relationship (i.e., $\text{Log}_{10}\ \text{SIR} = \log_{10} a + b . \log_{10} \text{SL}$), a least squares regression was performed to obtain a simple allometric equation ($\text{SIR} = a . \text{SL}^b$) for SIR relative to shell length (SL, mm). For the SIR-SL regression, the mean shell length and the pooled live weight of, and the lettuce consumed by, the set of snails in each feeding aquarium were employed, whereas for snails bigger than 15 mm the individual values of these variables were used. In order to allow comparisons with previous studies and to standardize food availability in growth efficiency experiments, similar allometric models were built for LC-LW and for LC-SL.

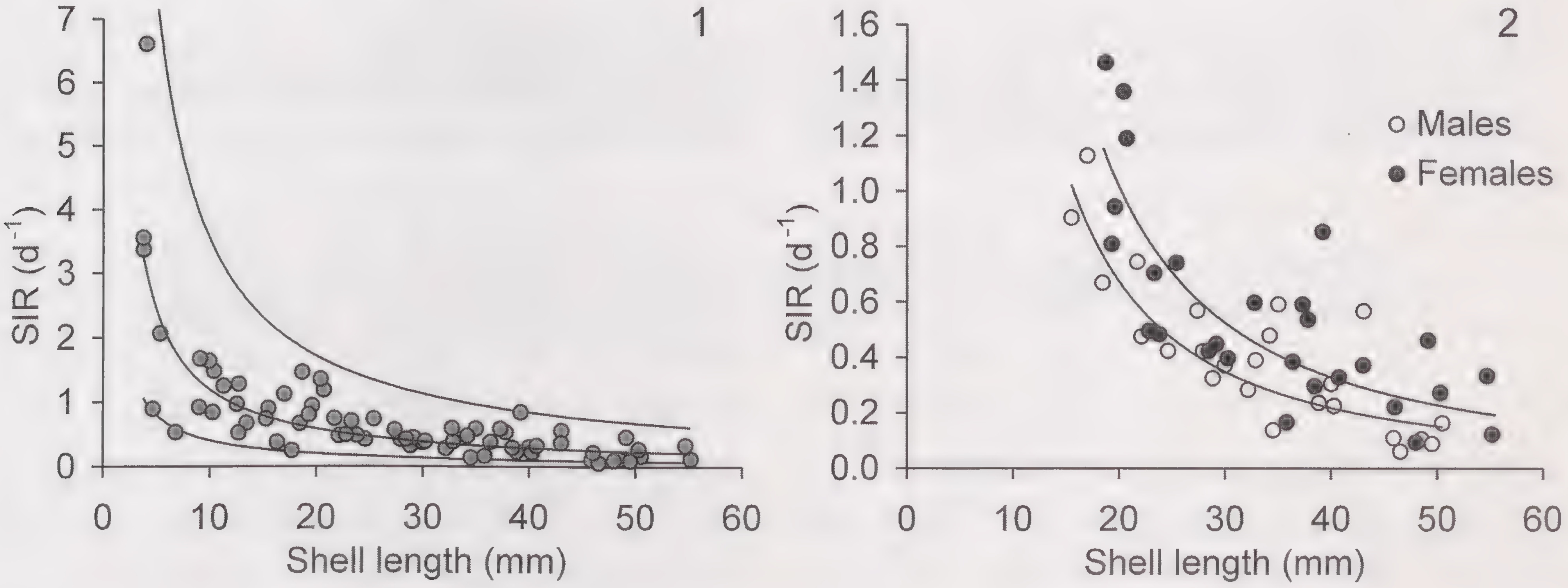
Experiment 2: Growth Efficiencies

In this experiment, the efficiency of conversion of food to body mass was analyzed under different levels of relative food availability. The whole experiment lasted 70 weeks, and the main goal was to study the reaction norm of age and size at maturity to food availability (Tamburi & Martin, 2009), but here mostly pre-reproductive data were considered. No death was recorded during the experimental period.

Hatchlings from a single egg mass laid by a laboratory female that had been coupled during all her life with the same male, were reared during three weeks in a 20 l aquarium. Seventy two of these full-sib snails were then randomly selected (shell length: 4.96 mm ± 0.056, mean ± SE, n = 25), and thereafter reared in isolation in 3 l aquaria under seven levels of relative food availability (FA%: 100, 87, 73, 60, 47, 33 and 20%). The expected daily amount of drained fresh lettuce ingestion by a snail of a given size (FA100%, g) was calculated using the LC-SL equation obtained from the data of Experiment 1 (i.e., $\text{LC} = \text{FA}100\% = 0.0033 . \text{SL}^{1.9322}$, $R^2 = 0.86$, n = 70).

Each Monday water was changed, aquaria were cleaned and their position within the rearing chamber was randomly changed. Snails were carefully observed to try to detect their sex as early as possible, and the shell length (SL) of each snail was measured as described in Experiment 1 (using a micrometric lens up to the sixth week of the experiment or with a caliper afterwards). The weekly amount of lettuce for each FA% level was calculated according to the mean shell length of males and females assigned to it, provided in two half-doses on Tuesdays and Fridays. On Mondays, the snails were not fed because this day was reserved in the experimental schedule for coupling with mature snails (reared independently for the maturity study). From the sixth week onwards, the live weight (LW, g) of each snail was measured at four or five weeks intervals.

To investigate the growth efficiencies at the same age, food conversion efficiencies (FCE) were calculated as $\text{FCE}_{i,f} = (\text{LW}_f - \text{LW}_i) . \text{AF}_{i,f}^{-1}$, where LW_i and LW_f are live weights at i^{th} and f^{th} week and $\text{AF}_{i,f}$ (g) is the accumulated weight of lettuce consumed in the same period. Except in the case of some males of the FA100% level during some weeks, the offered ration was to-



FIGS. 1, 2. Scatter-plots of specific ingestion rate (SIR) vs. shell length in *P. canaliculata* (curves were generated through the appropriate simple allometric models). FIG. 1: All snails (outer curves represent 95% prediction intervals); FIG. 2: Only differentiated snails.

TABLE 1: Two-way ANOVAs for food conversion efficiency (FCE) in *P. canaliculata* with sex and food availability (FA%) as main factors, calculated for the 0–6, 6–10, 10–14 and 14–19 weeks intervals (#: one-way ANOVAs for females only).

FCE	Sex	FA%	Sex x FA%
0–6	$F_{1,55} = 0.439$ $p = 0.511$	$F_{6,55} = 2.300$ $p = 0.047$	$F_{6,55} = 0.527$ $p = 0.785$
6–10	$F_{1,55} = 13.000$ $p < 0.001$	$F_{6,55} = 52.860$ $p < 0.001$	$F_{6,55} = 5.287$ $p < 0.001$
10–14 #	- -	$F_{4,21} = 7.419$ $p < 0.001$	- -
14–19 #	- -	$F_{4,19} = 20.360$ $p < 0.001$	- -

tally consumed, so AF_{if} was calculated as the sum of offered rations.

To investigate the growth efficiencies independently of size, the accumulated amount of food consumed to reach a given shell length (AFC, g) was calculated at 1mm intervals for shell lengths between 13 and 22 mm. For each snail, the moment at which a given shell length is attained (ASL, mm) was obtained by lineal interpolation between shell lengths at two successive weeks. The amount of food consumed since the previous Monday was also obtained by linear interpolation and added to the accumulated amount consumed up to the previous week.

The effect of food availability and sex on FCE and LW was investigated through two-way ANOVAs. Levene's test for homogeneity of variances was not rejected for FCE, while a Log_{10} transformation was required for LW. AFC was explored through a repeated measures ANOVA, with sex and FA% as main factors and the attained shell length (ASL) as the repeated measure or within-subject factor. Due to a significant non-sphericity (Mauchly's W test), the degrees of freedom were corrected by Greenhouse-Geisser's Epsilon.

RESULTS

Experiment 1: Ingestion Rates

No significant differences among males, females and undifferentiated snails were found in the rate at which the specific ingestion rate (SIR) decreased with shell length (slope equality test: $F_{2,65} = 1.65$, $P > 0.199$). For the whole sample

TABLE 2: Two-way ANOVA for the amount of food consumed (AFC) in *P. canaliculata* with sex and food availability (FA%) as main factors and attained shell length (ASL, from 13 to 22 mm) as repeated measure.

	Source	SS	df	F	p
Within-subject	ASL	9415.2	9	2735.2	< 0.001
	ASL x Sex	66.704	9	19.378	< 0.001
	ASL x FA%	29.535	54	1.430	0.182
	ASL x Sex x FA%	31.863	54	1.543	0.139
	Error (ASL)	182.44	477		
Between-subject	Sex	45.827	1	6.267	0.015
	FA%	45.142	6	1.029	0.417
	Sex x FA%	35.565	6	0.811	0.566
	Error	378.54	53		

TABLE 3: One-way ANOVA for the amount of food consumed (AFC) in males of *P. canaliculata* with food availability (FA%) as main factor and attained shell length (ASL, from 13 to 22 mm) as repeated measure.

	Source	SS	df	F	p
Within-subject	ASL	5996.8	9	1296.9	< 0.001
	ASL x FA%	28.710	54	1.035	0.430
	Error (ASL)	129.47	252		
Between-subject	FA%	62.970	6	1.050	0.415
	Error	279.58	28		

of snails (Fig. 1), the SIR was inversely related to shell length (b= -1.029; CI95% limits: -1.21 and -0.84); snails 12 mm-long consumed their own live weight in lettuce by day while for snails 50 mm-long the daily consumption was only 20% of their weight. The allometric coefficients for lettuce consumed (LC) per day relative to live weight or shell length were b = 0.651 (CI95% limits 0.59 and 0.71; $R^2 = 0.857$) and b = 1.932 (CI95% limits: 1.74 and 2.12; $R^2 = 0.862$), respectively. For SIR, significant differences in elevation were detected between sexes (elevation equality test: $F_{1, 49} = 9.94$, $P < 0.003$), ingestion rates being 50% higher for females than for males of the same shell length (Fig. 2) (back-transformed adjusted means for a 32.3 mm-long snail: 0.462 d^{-1} and 0.312 d^{-1}, respectively).

Experiment 2: Growth Efficiencies

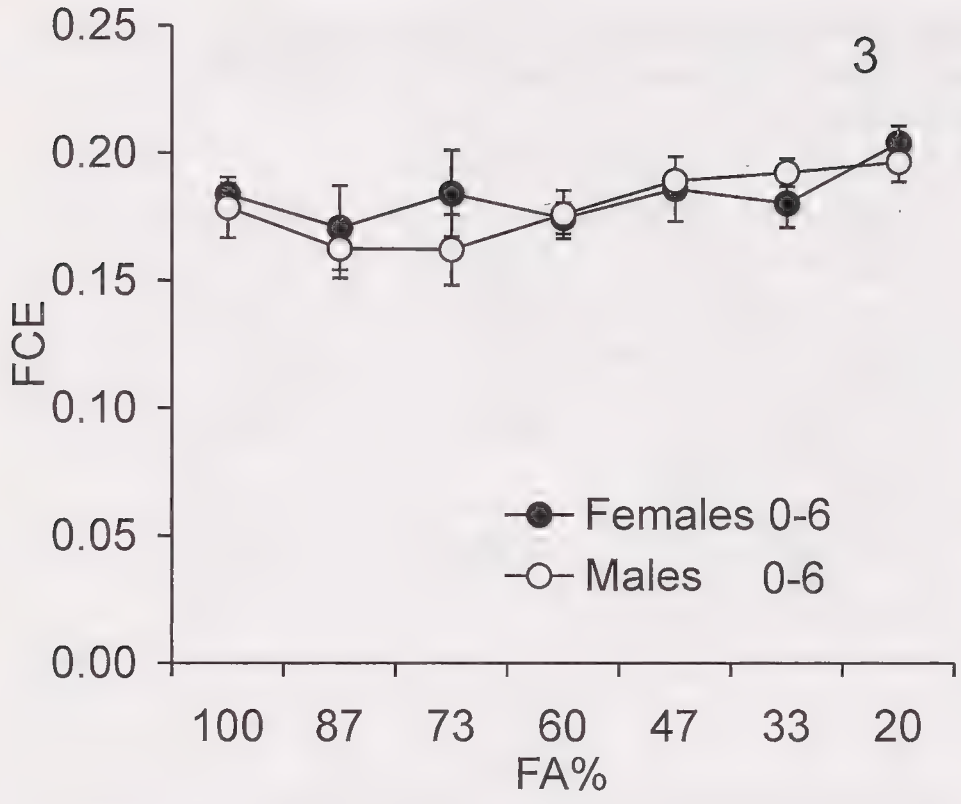
The food conversion efficiency during the first six weeks ($FCE_{0–6}$) showed only a marginally significant effect of food availability (Fig. 3, Table 1). During the following period, the $FCE_{6–10}$ showed a significantly different effect of food availability in each sex (Fig. 4, Table 1): no difference appeared between females fed at different FA% (one-way ANOVA: $F_{6, 27} = 1.51$, $p = 0.213$), while in males the FCE decreased significantly with FA% (one-way ANOVA: $F_{6, 28} = 11.31$, $p < 0.001$).

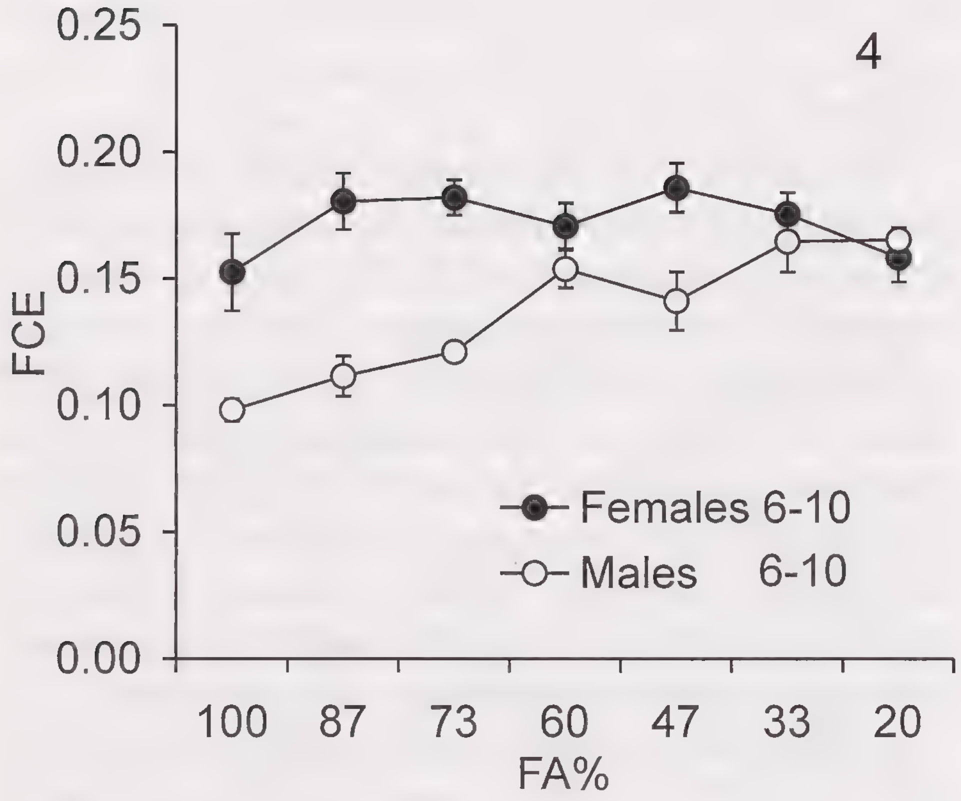
The males of all FA% levels and all the females from the 100 and 87 FA% levels were already mature by week 14 so FCEs were compared during the following two periods for the immature females of the remaining FA% levels. The FCE of these females was significantly affected by food availability, although a monotonically decreasing trend with FA% was apparent only at 14 to 19 weeks (Fig. 5, Table 1). For the same treatment and sex, the FCE values showed a general decreasing trend throughout the experiment.

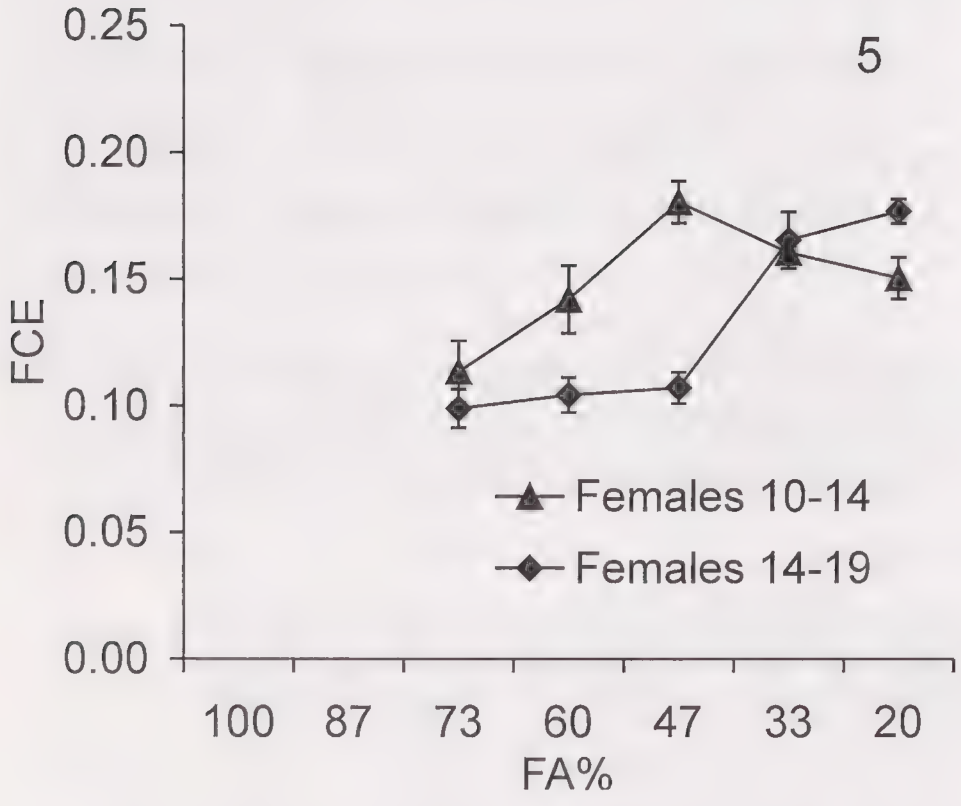
Differences in size among the snails of the different FA% levels were early noticeable in the experiment. The snails' weight ($\log_{10}LW$) at the sixth week was significantly affected by food availability ($F_{6, 55} = 293.81$, $p < 0.01$) but no inter-sex differences were found ($F_{1, 55} = 0.457$, $p = 0.502$; FA% x sex: $F_{6, 55} = 0.567$, $p = 0.754$). By the tenth week, a significant effect of the sex of snails ($F_{1, 55} = 62.08$, $p < 0.01$) appeared on the already significant effect of food availability ($F_{6, 55} = 892.47$, $p < 0.01$), being stronger in treatments of higher FA% (FA%

TABLE 4: One-way ANOVA for the amount of food consumed (AFC) in females of *P. canaliculata* with food availability (FA%) as main factor and attained shell length (ASL, from 13 to 22 mm) as repeated measure.

	Source	SS	df	F	p
Within-subject	ASL	3686.05	9	1739.64	< 0.001
	ASL x FA%	34.150	54	2.686	0.009
	Error (ASL)	52.970	225		
Between-subject	FA%	26.910	6	1.039	0.424
	Error	107.95	25		







FIGS. 3–5. Food conversion efficiency (FCE, mean ± SE) for pre-reproductive males and females of *P. canaliculata* under different food availabilities (FA%). FIG. 3: 0th to 6th week interval; FIG. 4: 6th to 10th week interval; FIG. 5: 10th to 14th and 14th to 19th week intervals (only for females that have not commenced egg laying).

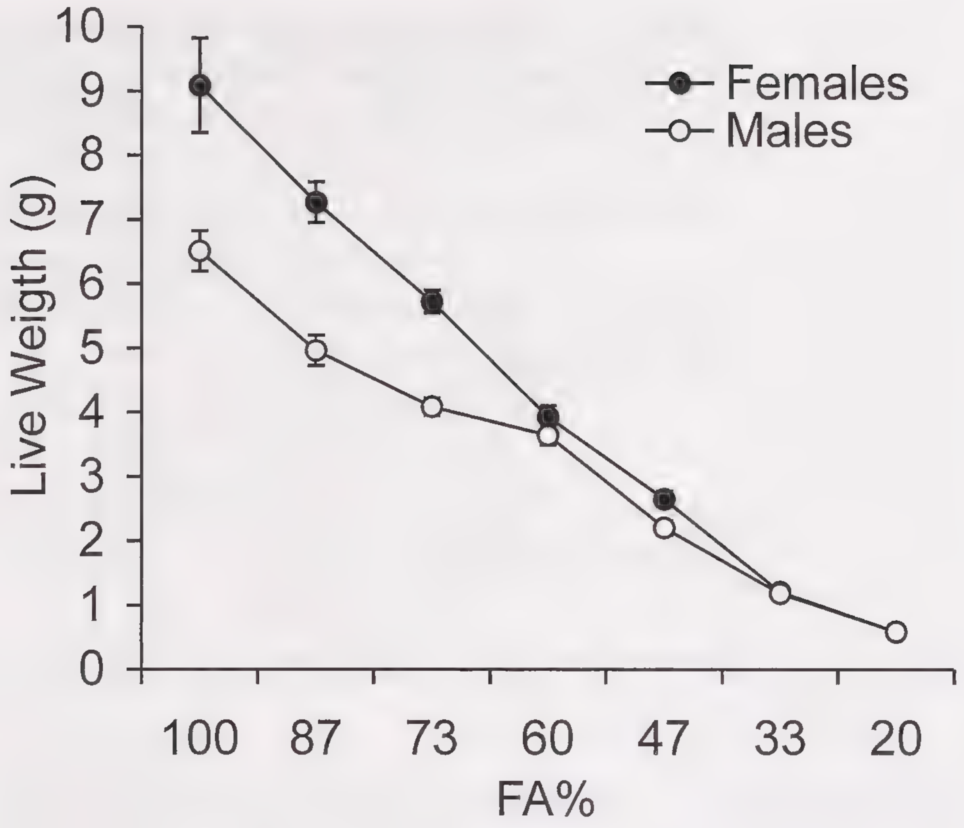
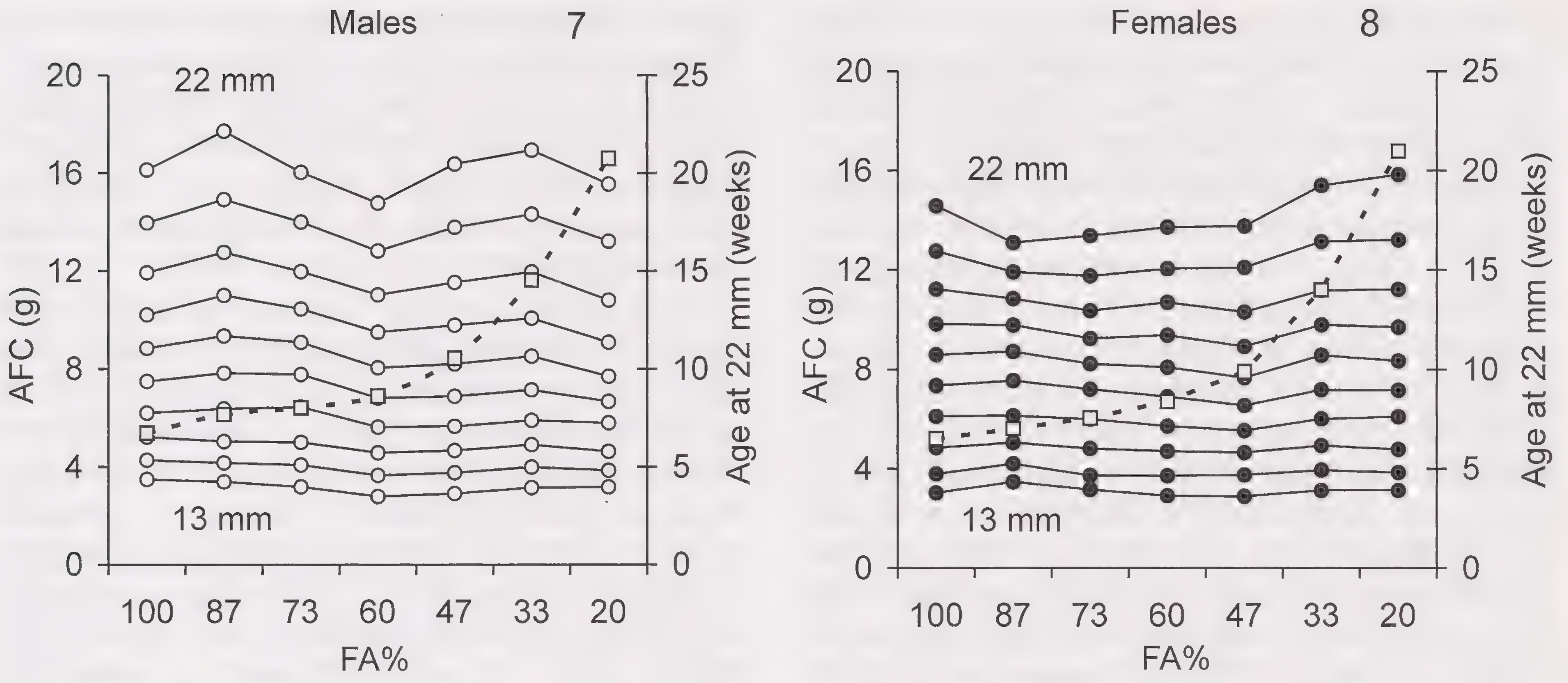


FIG. 6. Live weight (LW, mean ± SE) for both sexes of *P. canaliculata* at 10th week of rearing under different food availabilities (FA%).

x sex: $F_{6,\,55} = 6.59$, $p < 0.01$); females were increasingly heavier than males as food availability increased (Fig. 6). These same trends continued as long as the experiment ran and were also very similar when shell length was considered as an indicator of size.

A preliminary within-subject analysis using the whole data set of the amount of food consumed to attain a certain size (AFC) showed a significant effect of shell length and a significant interaction between shell length and sex (Table 2); the interaction between shell length and FA% and the second order interaction (ASL x FA% x sex) were both non-significant. The between-subject analysis of the same data set detected only a significant effect of sex (Table 2), with males requiring an increasingly higher amount of food than females to attain a certain size (Figs. 7, 8). Consequently, the analysis was split by sex to investigate in detail the effect of FA%.

The within-subject analysis for males showed only a significant effect of shell length while the between-subject analysis detected no differences between the FA% levels in the amount of food required to attain a given size (Fig. 7, Table 3). The same analyses for females showed a significant effect of shell length but also a significant interaction with the FA% levels that probably masked the differences among FA% levels in the between-subject analysis (Table 4); a noticeable effect of food availability on AFC appeared only in the late measurements of the



FIGS. 7, 8. Amount of food consumed (AFC) to attain a given shell length (entire lines, from 13 to 22 mm at 1 mm intervals) for *P. canaliculata* snails under different food availabilities (FA%; white squares represent the mean age of each FA% level when a shell length of 22 mm was attained). FIG. 7: Males; FIG. 8: Females.

lowest FA% (Fig. 8). The time required to attain a certain size (i.e., 22 mm) increased more than three fold with a decrease of 80% in trophic availability; males from the FA% levels of 20 and 33% were the only ones already showing copulatory behavior at this size, after attaining shell lengths of 15 and 17 mm, respectively.

DISCUSSION

Both ontogenetic and sexual differences were found in the specific ingestion rates of *Pomacea canaliculata* snails; this rate decreased inversely with shell length and was higher for females than for males of comparable size. Conversion efficiencies decreased with age in both sexes, the decrease being noticeable earlier in males than in females and greater at high than at low food availability levels; however, the effect of food availability almost disappeared when the effect of size was removed.

The specific ingestion rate decreases with shell length, from hatchlings to adult snails, when they are maintained under *ad libitum* palatable food. According to allometric models of herbivore feeding (Belovsky et al., 1999), which assume simple scaling growth, the food intake per unit time (C) would increase relative to body mass (M) with an allometry coefficient b = 0.66, and in turn body mass would increase to the cube of body length (b = 3). Therefore the allometry coefficient for the specific ingestion rate (SIR = C/M) relative to body length would be b = -1. In our study, the SIR was inversely related to shell length (b= -1.029), suggesting that a limitation in the specific ingestion rate arises as a result of the quadratic increase in the cross section of the digestive tube relative to the cubic increase in the individual biomass that it must serve. Allometric limitations of this kind seem common in apple snails, perhaps owing to shared ontogenetic patterns. Higher specific ingestion rates for small snails relative to big ones have been recently reported for *P. canaliculata* and *P. insularum* feeding on different plant species (including lettuce, Boland et al., 2008; Burlakova et al., 2009). In the Indian apple snail *Pila globosa*, specific feeding rates on *Ceratophyllum demersum* also decreased with size independent of food availability (Hannifa, 1982). Weight specific oxygen consumption rates (Freiburg & Hazelwood, 1977; Santos et al., 1987) also decrease with weight in *Pomacea* spp.; this could be in turn related to allometric limitations in gas exchange surfaces, an energy intake limited by size or probably both simultaneously. These allometric effects can explain in part the asymptotic decrease in growth rates in *P. canaliculata* (Estebenet & Martín, 2002).

Allometry coefficients for ingestion rates relative to weight show high variability in terrestrial snails and slugs (from 0.491 to 1.394;

Rollo, 1988). In contrast, the b-values (0.69 and 0.72) for the two freshwater gastropods listed by Rollo (1988) were close to the value obtained here for LC-LW (b = 0.651). Perhaps the higher variation in the relative ingestion rates of terrestrial mollusks is related to their marked daily rhythms of activity and to feeding opportunities that vary in an unpredictable way according to weather conditions. When food supplies are constant, *P. canaliculata* apparently feeds quite continuously both during the day and night (Estebenet & Martín, 2002; pers. obs.), although the activity level is higher during dark hours (Heiler et al., 2008). Jeschke & Tollrian (2005) observed that feeding times for the rams-horn apple snail *Marisa cornuarietis* (Linnaeus, 1758) are exceptionally high relative to those of a heterogeneous array of herbivores.

In our study, the amount of lettuce consumed increased quadratically (b = 1.932) for snails between 5 and 55 mm of shell length. Cazzaniga (1981) found that absolute ingestion rates for *P. canaliculata* snails feeding on *Chara vulgaris* increase monotonically between 42.5 and 62.5 mm of shell length but decreased for bigger snails (up to 72.5 mm). Due to the asymptotic growth pattern of this species (Estebenet & Martín, 2002), it is likely that the largest snails were disproportionately older and consequently already reaching senescence.

Females of *P. canaliculata* had a specific ingestion rate that was 50% higher than that of males. This could be related to the relatively smaller mid-gut gland of the males (61–72% of that of females; Vega et al., 2006), in which a greater portion of the visceral mass is occupied by the gonad, probably imposing a restriction in the amount of food that can be processed by unit time.

In our study, the males of *P. canaliculata* showed lower growth efficiencies than females, probably resulting from a higher waste of undigested food related to their smaller mid-gut gland, which in turn results in lower food assimilation. An alternative explanation would be a smaller fraction of assimilated resources devoted to growth in males (e.g., due to higher basal metabolic rates or to higher activity rates). However, this seems unlikely since specific oxygen consumption rates showed no intersexual differences in other *Pomacea* spp. (Freiburg & Hazelwood, 1977; Santos & Mendes, 1981), and in the particular case of *P. canaliculata* no sexual differences have been recorded in the lung ventilation frequency (Seuffert & Martín, 2009). It is worth mentioning that the mid-gut gland of females of *P. canaliculata* contains a 2.4-times higher concentration of a putative cyanobacterial endocytobiont than that of males (Koch et al., 2006; Vega et al., 2006), although their eco-physiological significance is far from being understood (secretion of proteases has been suggested; Godoy et al., 2005).

Even during pre-reproductive life, conversion efficiencies (FCE) were higher in females than in males, mostly due to its earlier decrease in the latter, especially at high food availabilities. Again, this could be explained by the smaller mid-gut gland of males owing to a greater portion of the visceral mass being taken up by the gonad (Vega et al., 2006). Coincidentally, in most males at the highest FA% levels (100-47%), the testicle was already observable through the shell by the second week of the experiment, while in those of the lowest levels (FA33-20%), the testicles were detected only at the fourth week and were clearly smaller (Tamburi & Martín, 2009).

The decrease in conversion efficiencies (FCE) along Experiment 2 was more important and earlier at high food availability levels. This generalized decline could be explained by the ageing of snails, by allometric limitations with size increase, or by the combination of both processes during growth. Ageing does not seems to be the main cause, since the efficiencies (AFC) estimated at the same size were the same across the different food availability levels, which differed greatly in the age necessary to attain a given shell length (Figs. 7, 8; Tamburi & Martín, 2009). The higher efficiencies (FCE) of snails under low food availabilities is probably due to the fact that they remain small and hence there is a lower decrease in the area of food absorption relative to the volume that must be nourished. However, the influence under deprived conditions of a higher absorption rate (e.g., due to higher retention times in the gut) or an increase in autocoprophagy cannot be ruled out. Haniffa (1982) found that absorption efficiencies in *P. globosa* also decreased with food availability.

Conversion efficiencies (FCE) for *P. globosa* (estimated from absorption and net conversion efficiencies in Table 1 in Haniffa (1982)) showed no effect of food availability and size on the range of food availabilities at which snails were able to grow. Food conversion rates (FCR, the inverse of our FCE) in *Pomacea bridgesii* (Reeve, 1856) were not affected by feeding levels ranging from 2% to 6% of snail live weight and a slightly higher efficiency was detected in small snails (Mendoza et al., 1999); food conversion rates in this study decreased with food crude protein contents ranging between 15% and 40% but Ramnarine (2004) found no such effect for *Pomacea urceus* (Müller, 1774) within the same range. Rearing *P. canaliculata* snails on a paper diet for 60 days reduces the mass of the mid-gut gland of males and females relative to those fed on a mixed diet (Vega et al., 2005), and this could affect their efficiency. Apparently size and food quality are important in determining food conversion efficiencies in apple snails but feeding level is not; the influence of sex, another important factor according to our results, has not been considered in previous studies.

The possibility of sexual dimorphism in feeding rates has not been considered in recent studies dealing with the subject in *P. canaliculata* and *P. insularum* (e.g., Peña & Pocsidio, 2007; Boland et al., 2008; Burlakova et al., 2009). Even if the sex ratio is balanced, the random sampling of snails to be subjected to different treatments can result in biased sex ratios in them, especially if samples are small; the sexually dimorphic feeding rates can greatly increase the variability of estimations or bias the means of certain treatments. The highly biased sex ratios reported in *P. canaliculata* egg masses and families (Yusa, 2007) can confound inter-study comparisons if sex ratio is not portrayed in the results.

Carlsson & Brönmark (2006) demonstrated that small *P. canaliculata* snails have higher foraging abilities than large ones, a conclusion strongly supported by our results, and suggested that this may also explain, through resource depletion, their higher competitive ability in terms of size increase. Our results indicate that size-dependent efficiency can also play a role in the competitive dynamics of *P. canaliculata* populations. Grazing would be more intense, and at the same time more selective (Carlsson & Brönmark, 2006), during the recruitment period, a fact that could affect macrophyte replacement or depletion (Tamburi & Martín, 2009), depending on the phenological cycle of palatable and unpalatable macrophytes in each waterbody.

According to our data, 103 newborn snails (5 mm long) would consume the same amount of lettuce, or vegetation, as one mature snail 55 mm long. One female can lay dozens of egg-masses composed of hundreds of eggs in one reproductive season (Estebenet & Martín, 2002; Martín & Estebenet, 2002), and at the end of it small snails comprise the majority of population in paddy fields (Wada & Matsukura, 2007). This strongly supports the predictions of Carlsson & Brönmark (2006) and Boland et al. (2008) that small snails, albeit inconspicuous, can be responsible as a group for a great part of the damage caused by apple snails in aquatic crops and natural wetlands, at least during the reproductive season.

The males of *P. canaliculata* have been shown to have lower growth rates than females (Estebenet & Cazzaniga, 1998; Tanaka et al., 1999; Estebenet & Martín, 2002; Estoy et al., 2002; Martín & Estebenet, 2002; Tamburi & Martín, 2009), and in this study they also exhibit lower ingestion rates and growth efficiencies relative to females. Size at maturity is very much less plastic than age at maturity in females, whereas the opposite is true in males (Tamburi & Martín, 2009). Male size is not relevant to the probability of copulation (Estebenet & Martín, 2002; Estoy et al., 2002) and has no effect on female reproductive output (Tamburi & Martín, 2009); in contrast, female size at maturity seems to be constrained by their high reproductive costs and the need to produce big, highly elaborate egg masses (Estoy et al., 2002; Tamburi & Martín, 2009). Consequently, a selection pressure to maximize growth rates would be expected in females, while this trait is unimportant in males. In males, the main pressure is probably to copulate as soon, and with as many females, as possible and to maximize the sperm transferred during each copulation, in order to have an advantage in sperm competition inside the female genitalia (Yusa, 2004; Burela & Martín, 2007). This probably means an increase in testicle size, which in turn reduces the mid-gut gland size and its capacity to process food, growth rate being reduced just as a by-product of the maximization of sperm production though probably without a collateral reduction in overall male fitness.

Contrary to many invertebrates (mostly arthropods) and other mollusks the energetic reserves of gastropod's embryos are located in the perivitelline fluid that surrounds egg-cell and not in the egg-cell itself (Heras et al., 1998, 2007). In the case of *P. canaliculata*, there seems to be a strong competition for space within the visceral mass between the food digestion-assimilation and the reproductive functions. In females, the organs that provide the eggs with the necessary reserves and protective structures (Catalán et al., 2002, 2006) cannot be accommodated inside the visceral mass without detriment to the digestive-assimilative functions performed by the mid-gut gland. This constraint has probably promoted the progressive assignment of these functions to the parts of the oviduct located outside the visceral mass, a trend that is widespread among gastropods (Fretter, 1984; Geraerts & Joose, 1984; Barker, 2001).

Our growth efficiencies results on *P. canaliculata*, albeit covering only the pre-reproductive phase, explain in part why females tend to grow to be larger than males (Estebenet & Cazzaniga, 1998; Estoy et al., 2002; Estebenet & Martín, 2003). The energetic budget probably changes at maturity for both sexes (Eleutheriadis & Lazaridou-Dimitriadou, 1995), but apparently not enough to hide the dimorphic pattern already expressed, which is probably due to the asymptotic decline in growth rates during their lifetime. This pattern is probably reinforced by the fact that after the beginning of testicular growth, males show a lower ingestion rate than females, and this is maintained during the reproductive lifespan. Bigger female sizes have also been reported for other species of ampullariids (Demian & Ibrahim, 1972; Burky, 1974; Keawjam, 1987; Lum-Kong & Kenny, 1989; Perera & Walls, 1996). Considering that testicle size and its occupancy of the visceral mass are higher than that of the ovary in most apple snails (except perhaps in *Afropomus balanoideus* (Gould, 1850); Berthold, 1988, 1989; Cowie et al., 2006), it is feasible that dimorphic ingestion rates and growth efficiencies are the cause of this widespread dimorphic pattern.

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NORTH PACIFIC SPECIES OF THE GENUS *SOLEMYA* LAMARCK, 1818 (BIVALVIA: SOLEMYIDAE), WITH NOTES ON *ACHARAX JOHNSONI* (DALL, 1891)

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ABSTRACT

North Pacific species of the family Solemyidae – *Petrasma pusilla* (Gould, 1861); *Solemya valvulus* Carpenter, 1864; *Solemya panamensis* Dall, 1908; *Petrasma pervernicosa* (Kuroda, 1948); *Solemya reidi* Bernard, 1980; and *Solemya tagiri* Okutani, Hashimoto & Miura, 2003 – and the type species of subgenera of the genus *Solemya* Lamarck, 1818, are re-examined. Five species of the genus *Solemya* are recognized from the North Pacific: *Solemya* (*Solemya*) *pusilla* (Gould, 1861); *Solemya* (*Solemya*) *valvulus* Carpenter, 1864; *Solemya* (*Solemya*) *tagiri* Okutani, Hashimoto & Miura, 2003; *Solemya* (*Petrasma*) *panamensis* Dall, 1908; and *Solemya* (*Petrasma*) *pervernicosa* Kuroda, 1948. The gutless *S. reidi* from the northeastern Pacific is synonymized with *S. pervernicosa* from the northwestern Pacific. *Solemya pervernicosa* was found in the Sea of Okhotsk and in the Sea of Japan near the coast of Sakhalin Island at the 300–800 m depth and was not previously recorded for Russia. A study of the materials of *Acharax johnsoni* (Dall, 1891) provided additional data on its distribution and ecology in the Pacific Ocean. This species was found in the Sea of Okhotsk (depth 370–1,515 m), as well as in the Monterey Canyon and Peru-Chile Trench (depth 3,040–4,200 m) in cold methane seep areas. The results of a comparative analysis of subgenera and species of the genus *Solemya*, descriptions of North Pacific species of the genus *Solemya*, and additional data on the distribution and ecology of *A. johnsoni* are presented.

Key words: *Solemya, Acharax,* systematics, morphology, distribution, cold seeps, Pacific Ocean.

INTRODUCTION

Representatives of the family Solemyidae are widely distributed in the North Pacific. To date, eight species of the Solemyidae are known for the North Pacific. On the coast of Japan, five species of this family have been reported: *Acharax johnsoni* (Dall, 1891), *Acharax japonica* (Dunker, 1882), *Petrasma pervernicosa* (Kuroda, 1948), *Petrasma pusilla* (Gould, 1861), and *Solemya tagiri* Okutani, Hashimoto & Miura, 2003 (Habe, 1977; Okutani, 2000; Okutani et al., 2003). In the northeastern Pacific, four species have been recorded: *A. johnsoni, Solemya reidi* Bernard, 1980; *Solemya panamensis* Dall, 1908; and *Solemya valvulus* (Carpenter, 1864) (Bernard, 1983; Coan et al., 2000). Nevertheless, in the Pacific seas of Russia, despite the neighborhood of coastal waters of Japan and North America, only *A. johnsoni* was recorded in the Bering Sea (Cape Olyutorski, depth 600–800 m, fresh valves) (Kafanov & Lutaenko, 1997). Also, Sahling et al. (2003) reported on the finding of *Acharax* sp. on the northeast Sakhalin slope and at the greatest depths in the Derjugin Basin, Sea of Okhotsk.

Examination of the extensive original material of the bivalves of the Russian Pacific seas revealed a large species (shell length more than 60 mm) of the genus *Solemya* Lamarck, 1818, from the Sea of Okhotsk and the northern Sea of Japan. Species of this genus have not been previously listed in the fauna of Russia (Scarlato, 1981; Kantor & Sysoev, 2005). Attempts to identify this species showed that identification of solemyid species is generally difficult because of large similarity of the form, proportions, and external and internal morphology of the shell, as well as because of the lack of important diagnostic features of the bivalves, such as distinct shell sculpture, hinge teeth, and pallial sinus. Moreover, the original and subsequent descriptions of most of North Pacific *Solemya* species are very brief, either with no or few and insufficiently detailed figures and illustrations.

This also hinders identification of species and their comparative analysis. Among all species of the Solemyidae recorded in the North Pacific, *S. reidi* was studied in greatest detail. Morphology of the shell, anatomy, ecology, and metabolic peculiarities of this species and its intracellular symbiotic bacteria have been fairly well studied by several authors (Bernard, 1980; Reid, 1980; Felbeck, 1983, 1985; Powell & Somero, 1985, 1986; Anderson et al., 1987, 1990; Gustafson & Reid, 1988a, b; O'Brien & Vetter, 1990; Lee et al., 1992; Wilmot & Vetter, 1992; Cary, 1994; Valentich-Scott, 1998; Coan et al., 2000). However, Bernard (1980), in describing *S. reidi*, erroneously placed it in *Solemya s. str.* (Taylor et al., 2008), and the subsequent authors did not change its taxonomic position (Valentich-Scott, 1998; Coan et al., 2000). Unlike *S. reidi*, such North Pacific species as *P. pervernicosa, S. panamensis, P. pusilla*, and *S. valvulus* have been much less studied. The description of *P. pervernicosa* was given in Japanese, and it was illustrated with a photograph of the right valve of the holotype (Kuroda, 1948). Later, a few authors provided only very brief descriptions of this species, sometimes duplicating their earlier description and photograph (Kira, 1959; Okutani et al., 1988; Okutani, 2000). Dall (1908a) gave a description of *S. panamensis* without illustrations. Much later, only Bernard (1980) provided a schematic drawing of the hinge of this species. The original and few subsequent descriptions of *P. pusilla* and *S. valvulus* are very brief, often fragmentary, and insufficiently illustrated (Gould, 1861; Carpenter, 1864; Dall, 1908a; Habe, 1977; Bernard, 1980; Kuznetsov et al., 1990; Coan et al., 2000; Okutani, 2000). Moreover, comparative analysis of *Solemya* species from the Russia Pacific seas showed that there is a confusion in the systematics of the Solemyidae from the North Pacific. Taylor et al. (2008) noted an analogous situation in Australian representatives of the Solemyidae. Having examined in detail Australian solemyids and the type species of genera and subgenera of the Solemyidae, Taylor et al. (2008) pointed to mistakes in the diagnoses of some subgenera of *Solemya* and redefined the recognized world genera and subgenera of the Solemyidae on the basis of characters ligament and features of the posterior aperture.

Thus, the identification of *Solemya* species found in the Pacific seas of Russia necessitated a more thorough examination of other species of the Solemyidae from the northern Pacific Ocean, including the type species of the genera and subgenera of this family.

In this paper, I present the results of a comparative analysis of shell morphology and descriptions of some species of the genus *Solemya* from the North Pacific supplemented by new data on their shell morphology and geographical distribution. Also, I present additional data on the distribution and ecology of *A. johnsoni* obtained from the study of the material of *Acharax* sp. from the Derjugin Basin, Sea of Okhotsk, which was previously examined by Sahling et al. (2003), and additional material of this species from this region and other areas of the Pacific Ocean.

MATERIALS AND METHODS

Material Studied

The material of *Solemya* species from the Sea of Okhotsk on the southeastern coast of Sakhalin Island and the Sea of Japan near the western coast of Sakhalin Island was collected by the Japanese research and commercial expedition with Russian observer (SakhRIFO scientific program, June–August 1997, fishing-boat "Taihei-Maru-106", Sea of Okhotsk), and Russian research and commercial expedition (SakhRIFO scientific program, November–December 2003, fishing-boat "Vulkanny", Sea of Japan). The material of this species from the Sea of Okhotsk was fixed with 4% formaldehyde and stored in 70% ethanol at the MIMB. The material from the Sea of Japan (fragments of shells) is stored dry at the IMB.

The material of *A. johnsoni* from the Sea of Okhotsk was collected by the IO RAS expedition to the Sea of Okhotsk (August–October 1949, cruise 2 of R/V "Vityaz") and the international expedition to Sakhalin Island (July–August 1998, cruise 28 of R/V "Akademik M. A. Lavrentyev"); from the Eastern Pacific, by the IORAS expeditions (September–December 1968, cruise 4 of R/V "Akademik Kurchatov"; August–October 1990, cruise 22 of R/V "Akademik Mstislav Keldysh"). The material of this species was stored in 70% ethanol and dry at the IO RAS.

Also, collection material and photos of the following taxa were used: *A. johnsoni* (ZMU); *P. pervernicosa* (NSMT); *P. pusilla* (NSMT); *S. reidi* (RBCM, CS, EMD); *Solemya australis* Lamarck, 1818 (AMS); *Solemya parkinsonii* Smith, 1874 (NMNZ); *Solemya velesiana* Iredale, 1931 (AMS); *Solemya terraereginae* Iredale, 1929 (AMS); *Solemya* sp. (IO RAS); *Solemya togata* (Poli, 1795) (NMW, photos by P. G. Oliver); *Solemya borealis* Totten, 1834 (MCZ, photos by A. J. Baldinger); *S. valvulus* (SBMNH, photos by P. Valentich-Scott); *S. panamensis* (SBMNH, photos by P. Valentich-Scott).

Shell Measurements

Figure 1 shows the shell morphology measurements. Shell length (L), anterior end length (A), height (H) were measured. The ratios of these parameters to shell length (A/L, H/L, respectively) were determined. Shell measurements were made using a caliper and an ocular micrometer with an accuracy of 0.1 mm.

Abbreviations

The following institutional abbreviations are used in the paper: AMS – Australian Museum, Sydney; CS – Columbia Science, Royston; BMNH – Natural History Museum, London; EMD – Environmental Monitoring Division, Los Angeles; IMB – A. V. Zhirmunsky Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok; IO RAS – P. P. Shirshov Institute of Oceanology, Russian Academy of Sciences, Moscow; MCZ – Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts; MIMB – Museum of the Institute of Marine Biology, Vladivostok; NMNZ – Museum of New Zealand Te Papa Tongarewa, Wellington; NMW – National Museums & Galleries of Wales, Cardiff; NSMT – National Science Museum, Tokyo; RBCM – Royal British Columbia Museum, Victoria; SakhRIFO – Sakhalin Scientific Research Institute of Fisheries & Oceanography, Yuzno-Sakhalinsk; SBMNH – Santa Barbara Museum of Natural History, Santa Barbara, California; USNM – United States National Museum of Natural History, Smithsonian Institute, Washington, D.C.; ZMMU – Zoological Museum of Moscow University, Moscow; ZMU – Zoological Museum of Far East State University, Vladivostok.

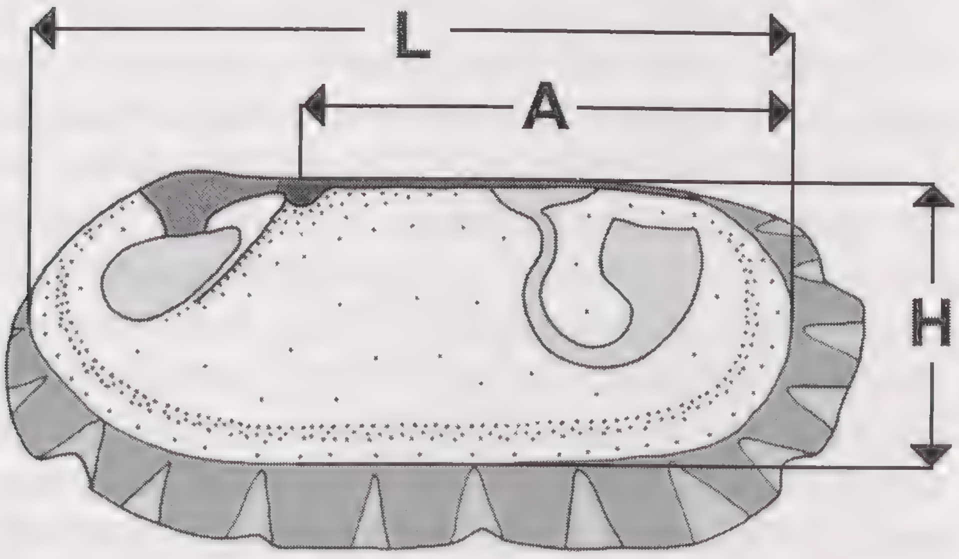


FIG. 1. Placement of shell measurements: L – shell length; H – height; A – anterior end length.

SYSTEMATICS

Family Solemyidae Gray, 1840
Genus *Solemya* Lamarck, 1818

Type Species – *Solemya mediterranea* Lamarck, 1818, = *Tellina togata* Poli, 1795; subsequent designation by Children, 1823.

Diagnosis

Shell thin, flexible, poorly mineralized, elongate-oval, inflated to compressed, inequilateral, equivalve. Beaks obscure, not prominent, near posterior end. Periostracum adherent, varnished, extending beyond shell. Surface with widely spaced weak radial ribs or furrows. Lunule and escutcheon absent. Ligament opisthodetic, internal, attached to chondrophore, sometimes with a lateral expansion in front of chondrophore. Chondrophore oblique, sometimes supported by a radial rib. Hinge plate weak, edentate. Adductor scars unequal; posterior scar small and impressed; anterior scar large, continuous with a pedal retractor scar.

The genus contains 16 living species.

Remarks

The genus *Solemya* was introduced with merely two species, *S. australis* and *S. mediterranea* (Dall, 1908a; Iredale, 1931; Bernard., 1980). The first listed, *S. australis*, was given as an example by Bowdich (1822), and this was incorrectly taken as a designation of a type species, such as by Dall (1908a). Children (1823) designated *S. mediterranea* as type. Nevertheless, a number of authors have accepted *S. australis* as the type species of *Solemya* (Dall, 1908a; Cox, 1969). Vokes (1955) showed that the first valid designation was *S. mediterranea* (= *T. togata*).

Dall (1908a) proposed subgeneric differentiation of *Solemya* species based on the type and structure of the ligament. He divided *Solemya* into three subgenera *Solemya* s.s., *Petrasma* Dall, 1908, and *Acharax* Dall, 1908. In Dall's (1908a) classification, the subgenus *Solemya* s.s. has an internal ligament with a lateral expansion in front of the chondrophore; the subgenus *Petrasma* also has an internal ligament, but without a lateral expansion in front of the chondrophore, and the subgenus *Acharax* has an external ligament. However, Dall (1908a) considered the type species of the genus *Solemya* to be *S. australis*, not

Solemya togata (Poli, 1795). Therefore, Dall's (1908a) diagnosis of *Solemya* s.s. was primarily made on the basis of the ligament structure of *S. australis*. Dall (1908a) included *S. togata* in the subgenus *Solemya* s.s. and indicated that in this species "… the anterior exposure of the internal part of the ligament is reduced to a narrow line directed backward to the upper anterior angle of the posterior adductor scar." In reality, unlike *S. australis*, *S. togata* lacks a lateral expansion of the internal ligament in front of the chondrophore (Taylor et al., 2008) and has instead only narrow strips of internal ligament under the chondrophore (Fig. 2). Since *S. togata* is the type species of the genus *Solemya*, the diagnosis of the subgenus *Solemya* s.s. should be modified according to features of the shell morphology of *S. togata*. However, some workers (Bernard, 1980; Coan et al., 2000), subsequently indicated *S. togata* to be the type species of the genus *Solemya* but retained Dall's (1908a) diagnosis of *Solemya* s.s.

Based on ligament structure and external shell morphology, Iredale (1931) introduced the genus *Solemyarina* Iredale, 1931, to accommodate two small Australian species, *S. velesiana*, and *S. terraereginae*, and designated *S. velesiana* as its type species. Later, Iredale (1938) included in the genus *Solemyarina* a subgenus *Zesolemya* Iredale, 1938, with *S. parkinsonii*, from New Zealand as its type species.

Cox (1969) placed *Zesolemya* in synonymy of *Solemyarina*, which he recognized as a subgenus of *Solemya*. He gave an accurate diagnosis of the subgenus *Solemya* s.s., describing the ligament and chondrophore of the type species *S. togata*. Also, Cox (1969) noted that *S. australis*, which some authorities have accepted as type species of *Solemya*, belongs to the subgenus *Solemyarina*. However, Lamprell & Healy (1998) returned *S. australis* to *Solemya* s.s.

Bernard (1980), probably adhering to Dall's (1908a) diagnosis of *Solemya* s.s., noted that *Solemya* s.s. has a lateral expansion of the internal ligament in front of the chondrophore. Relying on this diagnosis, he thought that the genus *Solemyarina* and the subgenus *Zesolemya* recognized by Iredale (1931, 1938) fell into *Solemya* s.s. and the genus *Solemya* contained only two living subgenera, *Solemya* s.s. and *Petrasma*. Japanese malacologists came to consider these to be full genera of the Solemyidae (Habe, 1977, 1981; Okutani et al., 1988; Tsuchida & Kurozumi, 1993; Higo et al., 1999; Okutani, 2000).

Coan et al. (2000) believed that the genus *Solemya* in the North Pacific comprised the subgenera *Solemya* s.s. and *Petrasma*, but their diagnoses of these subgenera repeated those of Bernard (1980). Their subgeneric differentiation was based not only on the morphology of the ligament, but also on the presence or absence of an internal rib supporting the chondrophore. In the opinion of Coan et al. (2000), the main character distinguishing *Solemya* s.s. is the presence of an internal, subumbonal expansion or lateral lobes of the ligament in front of the chondrophore and a radial rib supporting the chondrophore, with the species of the subgenus *Petrasma* having no internal lobes of the ligament in front of the chondrophore and nor a radial rib supporting the chondrophore. However, in reality, *S. togata* (type species of *Solemya* s.s.) has no internal lobes of the ligament in front of the chondrophore and nor a radial rib supporting the chondrophore, whereas *S. borealis* (type species of subgenus *Petrasma*) has a radial strengthening rib of chondrophore (Figs. 2, 3). In a recent work, Japanese malacologists (Okutani et al., 2003) have also begun to follow generic/subgeneric differentiation of the Solemyidae proposed by Bernard (1980) and Coan et al. (2000).

Taylor et al. (2008) thoroughly studied Australian solemyids and types of all previously recognized genera and subgenera of the Solemyidae. They revealed all main inaccuracies in the systematics of the Solemyidae made by previous authors. Based on ligament characters and features of the posterior aperture, they recognized the subgenera within the genus *Solemya* – *Petrasma* (type species *S. borealis*), *Solemyarina* Iredale, 1931 (type species *S. velesiana*) and *Zesolemya* Iredale, 1938 (type species *S. parkinsonii*), and additionally proposed a new subgenus *Austrosolemya* Taylor, Glover & Williams, 2008, with *S. australis* as its type species (Figs. 3–6). Taylor et al. (2008) described, in detail, the ligament, chondrophore and associated structures, and posterior aperture of all type species of the genera and subgenera of the Solemyidae. These features of type species provided the basis for generic and subgeneric differentiation of the Solemyidae. The characters of posterior aperture such as the number and arrangement of papillae were suggested as having some potential in the solemyid systematics. Thus, many previous authors (Dall, 1908a; Cox, 1969; Bernard, 1980; Coan et al., 2000), as a rule, split the genus *Solemya* into subgenera primarily on the basis of two characters, namely, the presence or absence of an expansion of the internal ligament in front of the chondrophore and ridges supporting the chondrophore. Taylor et al. (2008) included in the diagnosis of subgenera a complex of characters, including the form of the internal ligament, form of the expansion of the internal ligament in front of the chondrophore, form of the chondrophore, and form of the inner ribs strengthening the chondrophore.

The study of all Pacific species of *Solemya* and the type species of genera and subgenera of living Solemyidae shows that, in my opinion, all Pacific species of the genus *Solemya* recorded along the coasts of Asia and America may be referred to the subgenera *Solemya* s.s. and *Petrasma*. Also, as a result of detailed examination of the type species of solemyid genera and subgenera and the emendation of the diagnoses of *Solemya* s.s. and *Petrasma*, the taxonomic position of some Pacific species is herein changed.

Subgenus *Solemya* s.s.

Diagnosis

Internal ligament broad, subtriangular, sometimes with posterior extensions. Lateral expansion of ligament in front of chondrophore absent. Chondrophore not supported by a radial rib, not bounding anterodorsal margin of posterior adductor scar. Anterior end of posterior adductor scar not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore.

Remarks

The subgenus *Solemya* s.s. primarily differs from the subgenera *Solemyarina*, *Zesolemya*, and *Austrosolemya* in lacking a lateral expansion of internal ligament in front of the chondrophore (Figs. 2–6, Table 1). Although similar to many species of the subgenus *Petrasma* in having no lateral expansion of the internal ligament in front of the chondrophore, the subgenus *Solemya* s.s. differs from it in that the chondrophore is not supported by a radial rib bounding the anterior margin of the posterior adductor scar, and the anterior end of the posterior adductor scar is not impressed into the inner shell surface and does not adjoin the anteroventral margin of the chondrophore.

Solemya (*Solemya*) *pusilla* Gould, 1861
(Figs. 7–14)

Solemya pusilla Gould, 1861: 27.
Solemya (*Petrasma*) *pusila* Gould – Dall, 1908a: 364.
Petrasma pusilla (Gould, 1861) – Habe, 1977: 11, pl. 1, figs. 3, 4; Habe, 1981: 25; Tsuchida & Kurozumi, 1993: 2–3, pl. 1, fig. 1; Higo et al., 1999: 413; Okutani, 2000: 833, pl. 414, fig. 3.
Solemya (*Solemya*) *pusilla* Gould, 1861 – Bernard et al., 1993: 20.

Type Material and Locality

Holotype (USNM 24204), Hakodadi Bay (Hakodate, Hokkaido, Japan), 9.1 m, muddy bottom (Higo et al., 1999; P. Valentich-Scott, personal communication).

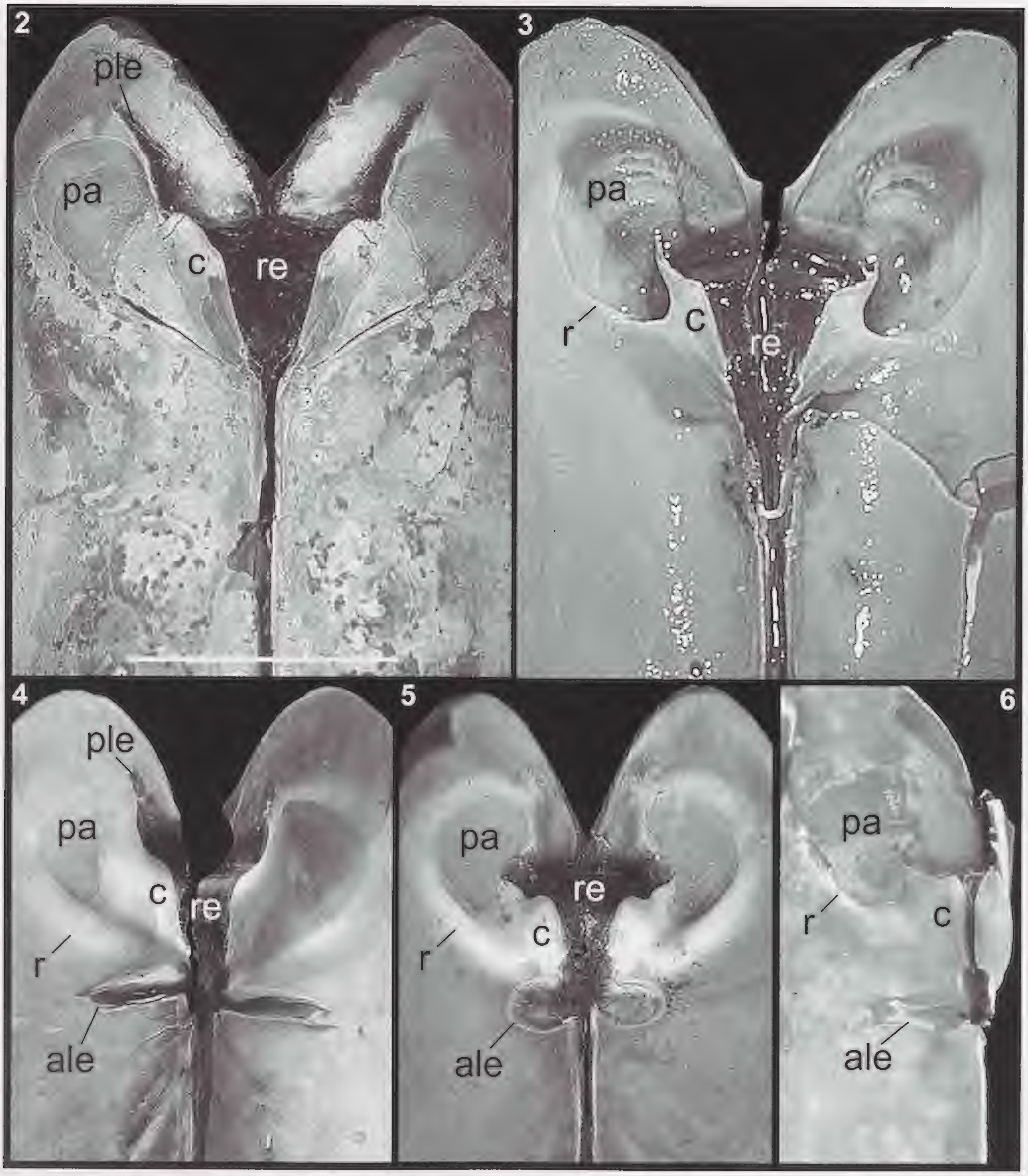
Material Examined

Four specimens (NSMT Mo 48815) and 3 specimens (NSMT Mo 76805), Loc. Tomioka, Amakusa Islands, Kyushu, Japan; 4 specimens (NSMT Mo 60259), Loc. Tsukumo Bay, Ishikawa Prefecture, Honshu, Japan. Total of 11 specimens.

Description

Exterior: Shell small (to 15 mm), elongate-oval, equivalve, strongly inequilateral, thin, flexible, poorly mineralized, semitransparent. Sculpture of very low, broad, widely spaced radial ribs in anterior part of shell and weak, fine ribs in central and posterior parts of shell. Periostracum thin, grayish-yellow, almost white in umbonal area, widely extending beyond shell margin. Beaks very small, obscure, not prominent, near posterior end. Anterior shell end gently rounded. Posterior shell end slightly angular, with a noticeable, short, radial, oblique flexure, corresponding to attachment of chondrophore to inner shell surface. Posterodorsal shell margin slightly convex in internal ligament area, forming a small, smooth, obtuse angle, smoothly transitioning to slightly angular posterior margin. Anterodorsal shell margin straight, parallel to ventral margin, smoothly transitioning to rounded anterior margin. Ventral margin straight.

Interior: Ligament thin, subtriangular, moderately broad, with short sharpened posterior extensions, attached to chondrophore, lacking



FIGS. 2–6. Ventral view of ligament and chondrophore of type species of subgenera of the genus *Solemya*. FIG. 2: *Solemya togata* (type species of *Solemya* s.s.), Mediterranean Sea (NMW, photo by P. G. Oliver); FIG. 3: *Solemya borealis* (type species of *Petrasma*), Cape Cod Bay, 5 mi. N of Barnstable MA., Atlantic Ocean, shell length 57.0 mm, (MCZ 302391, photo by A. J. Baldinger); FIG. 4: *Solemya parkinsonii* (type species of *Zesolemya*), N Douglas, Auckland, Tamaki Strait, Kawaka Bay, North Island, New Zealand, shell length 35.5 mm, (NMNZ M 274993); FIG. 5: *Solemya australis* (type species of *Austrosolemya*), 35°15.000'S, 137°0.000'E, South Australia, shell length 45.0 mm, (AMS C 085088); FIG. 6: *Solemya velesiana* (type species of *Solemyarina*), Port Jackson, Sydney (BMNH-95.3.7.17-19) (from Taylor et al., 2008, fig. 1C). ale, anterior lateral expansion; c, chondrophore; pa, posterior adductor scar; ple, posterior lateral extension; r, radial rib in front of posterior adductor scar; re, resilium. Scale bar = 5 mm.

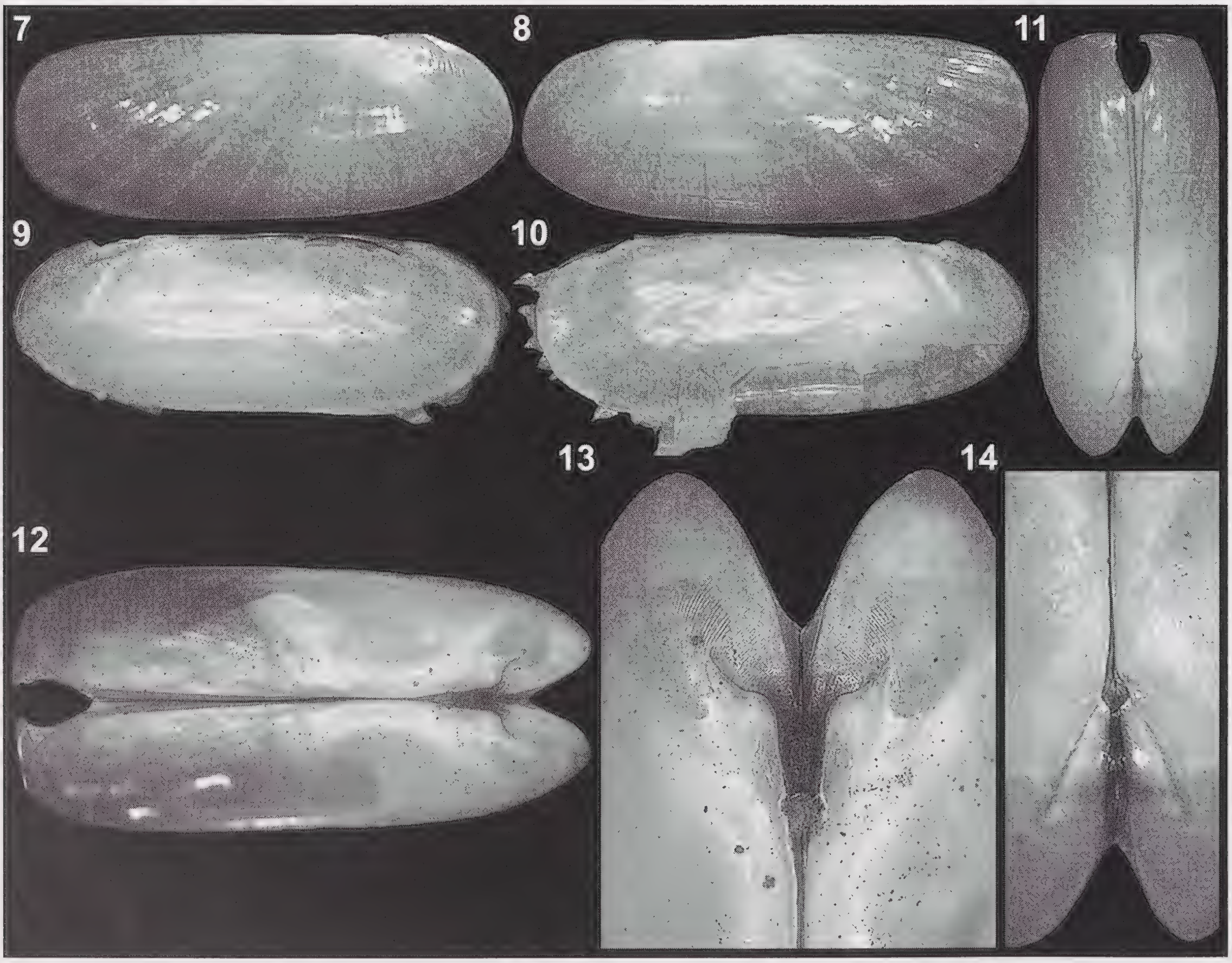
TABLE 1. Comparison of main morphological characters among subgenera of the genus *Solemya*.

Characters	*Austrosolemya*	*Petrasma*	*Solemya* s.s.	*Solemyarina*	*Zesolemya*
Resilium	Subtriangular, broad	Subtriangular, broad	Subtriangular, broad	Narrow	Subtriangular, broad, with narrow posterior extensions
Internal ligament expansion in front of chondrophore	Two large, oval, lateral lobes	Absent or small, irregular, sometimes elongate along shell dorsal margin	Absent	Heart-shaped, with two narrow, short, lateral striae	Two narrow, long, lateral striae
Internal radial rib in front of posterior adductor scar	Strong, broad, high, supporting anterior end of chondrophore	Week, narrow, low, supporting posterior end of chondrophore	Absent	Week, narrow, low, not supporting chondrophore	Weak, broad, low, not supporting chondrophore
Chondrophore	Short, thick, not bounding anterodorsal margin of posterior adductor scar	Short, thick, bounding anterodorsal margin of posterior adductor scar	Short, thin, not bounding anterodorsal margin of posterior adductor scar	Short, thin, not bounding anterodorsal margin of posterior adductor scar	Long, thin, not bounding anterodorsal margin of posterior adductor scar
Posterior adductor scar	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore	Impressed into inner shell surface, adjoining anteroventral margin of chondrophore	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore

an expansion in front of chondrophore, visible externally as sunken, small, brown, diamond-shaped button behind beaks. Chondrophore strong, long, thin, oblique, situated above posterior adductor scar, not bounding anterodorsal margin of posterior adductor scar. Internal radial rib in front of posterior adductor scar very weak, low, broad, not supporting chondrophore. Anterior adductor scar large, angular-oval, well impressed. Posterior scar smaller, ovate-triangular, well impressed, not adjoining anteroventral margin of chondrophore, extending behind chondrophore. Shell interior grayish, with weak, dense radial lines. Digestive tract present.

Comparisons

This species is easily distinguished from the Pacific species *S. australis, S. parkinsonii, S. pervernicosa, S. terraereginae,* and *S. velesiana* by the lack of an internal expansion of the ligament in front of the chondrophore (Table 2). In contrast to *S. panamensis* (Figs. 22–30), *S. pusilla* has sharpened posterior extensions of the resilium, the posterior adductor scar does not adjoin the anteroventral margin of the chondrophore, and the internal radial rib in front of the anterior margin of the posterior adductor scar does not support the chondrophore. *Solemya pusilla* is closest to *S. tagiri, S. valvulus,* and *Solemya moretonensis* Taylor, Glover & Williams, 2008. *Solemya pusilla* differs from *S. tagiri* in having an internal radial rib in front of the anterior margin of the posterior adductor scar, sharpened posterior extensions of the resilium, a longer chondrophore, and alimentary canal; from *S. valvulus* (Figs. 15–21), in having a radial rib in front of the anterior margin of the posterior adductor scar and that the posterior



FIGS. 7–14. *Solemya (Solemya) pusilla*, Loc. Tomioka, Amakusa Islands, Kyushu, Japan (NSMT-Mo 76805). FIGS. 7–10: Exterior and interior views of left and right valves, shell length 7.3 mm; FIGS. 11–14: Dorsal and interior views of both valves, ventral view of ligament and chondrophore, dorsal view of umbonal area, shell length 10.0 mm.

adductor scar extends behind the chondrophore. Unlike *S. moretonensis*, the posterior adductor scar of *S. pusilla* extends behind the chondrophore.

Distribution and Habitat

Solemya (*Solemya*) *pusilla* occurs in the western Pacific off Japan from the Kii Peninsula (Honshu, Pacific Ocean) and Seto Inland Sea to southern Hokkaido; in the Sea of Japan (Tsukumo Bay, Ishikawa Prefecture, Japan – NSMT Mo 60259); in the East China Sea (Amakusa Island, Kyushu, Japan – NSMT Mo 48815, 76805); in the Yellow Sea. This species has been recorded from the intertidal zone to 20 m, on sandy silt, silt with a smell of hydrogen sulphide (Habe, 1977, 1981; Kuznetsov et al., 1990; Bernard et al., 1993; Tsuchida & Kurozumi, 1993; Higo et al., 1999; Okutani, 2000).

Solemya (*Solemya*) *valvulus* Carpenter, 1864
(Figs. 15–21)

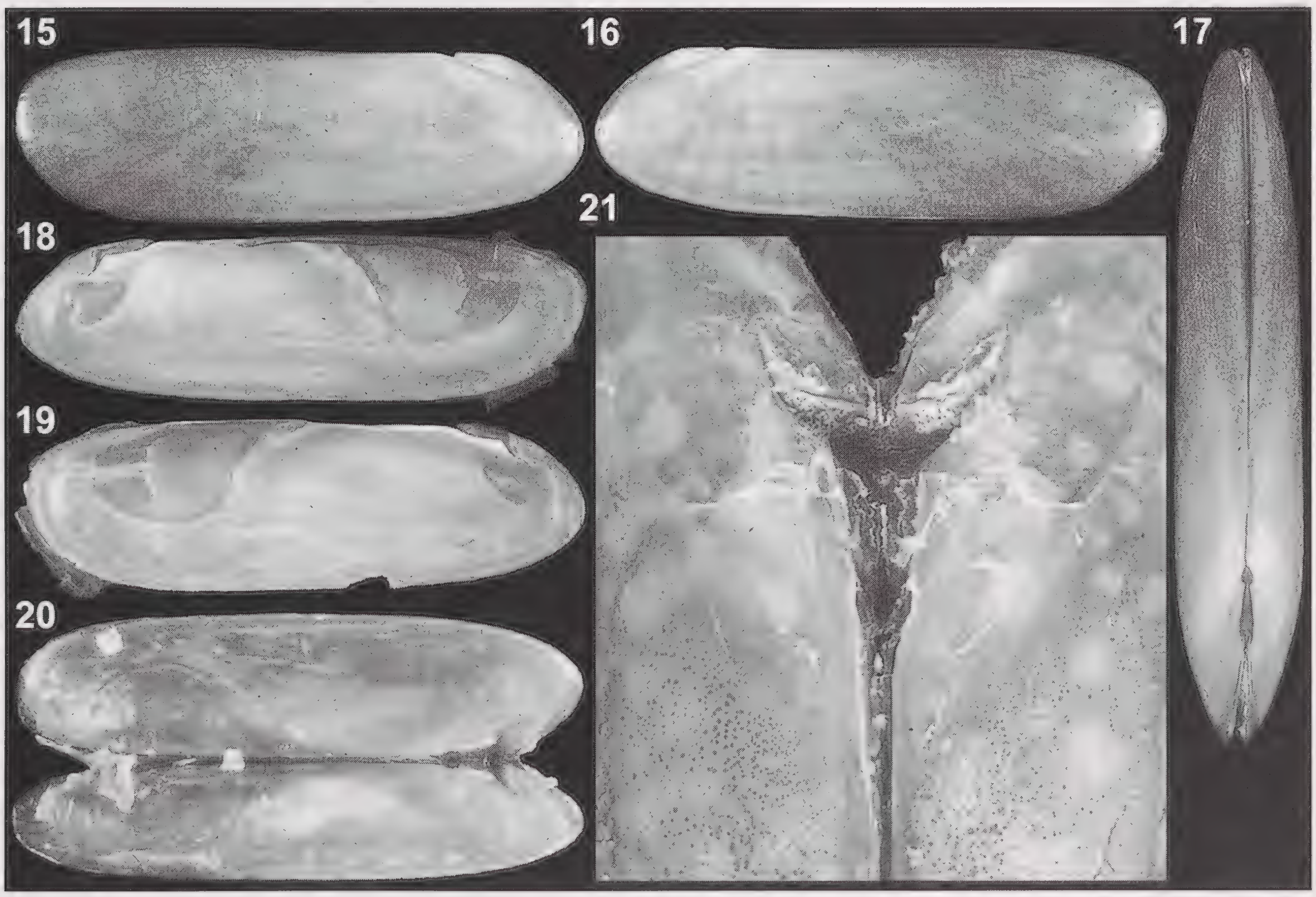
Solenomya valvulus Carpenter, 1864: 311.
Solemya (*Petrasma*) *valvulus* Carpenter – Dall, 1908a: 364.
Solemya (*Petrasma*) *valvulus* Carpenter, 1864 – Bernard, 1980: 18, fig. 3; Bernard, 1983: 9.
Solemya (*Petrasma*) *valvulus* (Carpenter, 1864) – Coan et al., 2000: 66, pl. 1.

Type Material and Locality

Type is lost. Cabo San Lucas, Baja California Sur, Mexico (Palmer, 1958; E. V. Coan & P. Valentich-Scott, personal communication)

Description

Exterior: Shell small (to 20 mm), elongate-oval, equivalve, strongly inequilateral, thin, flexible, poorly mineralized. Sculpture of very low, broad, widely spaced radial ribs, less expressed medially, and narrow, radial, thread-like ribs in space between broad ribs of anterior shell end.



FIGS. 15–21. *Solemya (Solemya) valvulus*, Bahia Santiago (19°06'24"N, 104°22'28"W), Colima, Mexico, Pacific Ocean (SBMNH 83479, photos by P. Valentich-Scott). FIGS. 15–17: Exterior view of left and right valves, dorsal view of both valves, shell length 17.75 mm; FIGS. 18, 19: Interior view of left and right valves, shell length 16.25 mm; FIGS. 20, 21: Interior views of both valves, ventral view of ligament and chondrophore, shell length 17.08 mm.

TABLE 2. Main differentiating characters of *Solemya* species from the Pacific Ocean.

Species	Maximum shell length, mm	Resilium	Internal ligament expansion in front of chondrophore	Chondrophore	Internal radial rib in front of posterior adductor scar	Posterior adductor scar	Alimentary system	References
Solemya (Solemya) pusilla	14.5	Subtriangular, broad, with very short, sharpened posterior extensions	Absent	Long, thin, not bounding anterodorsal margin of posterior adductor scar	Very weak, broad, low, not supporting chondrophore	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore, extending behind chondrophore	Present	Dall, 1908; Habe, 1977; Kuznetsov et al., 1990; Okutani, 2000
Solemya (Solemya) valvulus	20.0	Subtriangular, broad, with sharpened posterior extensions	Absent	Long, thin, not bounding anterodorsal margin of posterior adductor scar	Absent	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore, not extending behind chondrophore	Present	Dall, 1908; Coan et al., 2000
Solemya (Solemya) tagiri	23.8	Subtriangular, broad	Absent	Very short, thin, not bounding anterodorsal margin of posterior adductor scar	Absent	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore, not extending behind chondrophore	Absent	Okutani et al., 2003
Solemya (Petrasma) panamensis	47.0	Subtriangular, broad	Absent	Short, thin, bounding anterodorsal margin of posterior adductor scar	Week, narrow, low, supporting anterior end of chondrophore	Impressed into inner shell surface, adjoining anteroventral margin of chondrophore, extending behind chondrophore	Present	Dall, 1908; Coan et al., 2000
Solemya (Petrasma) pervernicosa	63.1	Subtriangular, broad	Oval or elongate-oval, often irregularly shaped, with short lateral rays	Short, thick, bounding anterodorsal margin of posterior adductor scar	Week, narrow, low, supporting posterior end of chondrophore	Impressed into inner shell surface, adjoining anteroventral margin of chondrophore, extending behind chondrophore	Absent	Kuroda, 1948; Bernard, 1980; Okutani et al., 1988; Valentich-Scott, 1998; Coan et al., 2000; Okutani, 2000

(continues)

(continued)

Species	Maximum shell length, mm	Resilium	Internal ligament expansion in front of chondrophore	Chondrophore	Internal radial rib in front of posterior adductor scar	Posterior adductor scar	Alimentary system	References
Solemya (Solemyarina) terraereginae	8.0	Narrow, with parallel dorsal edges of chondrophore	Heart-shaped, with two narrow, short, lateral striae	Short, thin, not bounding anterodorsal margin of posterior adductor scar	Week, narrow, low, supporting anterior end of chondrophore	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore, extending behind chondrophore	Present	Iredale, 1929, 1938; Taylor et al., 2008
Solemya (Solemyarina) velesiana	16.0	Narrow, with parallel dorsal edges of chondrophore	Heart-shaped, with two narrow, short, lateral striae	Short, thin, not bounding anterodorsal margin of posterior adductor scar	Week, narrow, low, not supporting chondrophore	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore, extending behind chondrophore	Present	Iredale, 1931; Lamprell & Healy, 1998; Taylor et al., 2008
Solemya (Zesolemya) parkinsonii	75.0	Subtriangular, broad, with long, rounded posterior extensions	Two narrow, long, lateral striae	Very long, thin, not bounding anterodorsal margin of posterior adductor scar	Weak, broad, low, not supporting chondrophore	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore, not extending behind chondrophore	Present	Smith, 1874; Dall, 1908; Owen, 1961; Beedham & Owen, 1965; Taylor et al., 2008
Solemya (Austrosolemya) australis	60.0	Subtriangular, broad	Two large, oval, lateral lobes	Short, thick, not bounding anterodorsal margin of posterior adductor scar	Strong, broad, high, supporting anterior end of chondrophore	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore, extending behind chondrophore	No data	Dall, 1908; Cox, 1969; Lamprell & Healy, 1998; Taylor et al., 2008
Solemya (s.l.) *moretonensis*	6.6	Subtriangular, broad, with short, sharpened posterior extensions	Absent	Long, thin, not bounding anterodorsal margin of posterior adductor scar	Very weak, narrow, low, not supporting chondrophore	Not impressed into inner shell surface, not adjoining anteroventral margin of chondrophore, not extending behind chondrophore	No data	Taylor et al., 2008

Periostracum thick, brown at anterior shell end and light brown or yellow at posterior shell end and umbonal area, widely extending beyond shell margin. Beaks very small, obscure, not prominent, near posterior end. Anterior shell end gently rounded. Posterior shell end slightly angular and narrowed, with a well expressed radial, oblique flexure, corresponding to attachment of chondrophore to inner shell surface. Posterodorsal shell margin slightly convex in internal ligament area, forming a small smooth, obtuse, angle, smoothly transitioning to slightly angular posterior margin. Anterodorsal shell margin straight, parallel to ventral margin, smoothly transitioning to rounded anterior margin. Ventral margin straight.

Interior: Ligament thin, subtriangular, moderately broad, with sharpened posterior extensions, attached to chondrophore, without an expansion in front of chondrophore, visible externally as sunken, small, brown, subtriangular button behind beaks. Chondrophore strong, long, oblique, situated above posterior adductor scar, not bounding anterodorsal margin of posterior adductor scar. Internal radial rib in front of posterior adductor scar absent. Anterior adductor scar large, angular-oval, feebly impressed. Posterior scar smaller, ovate-triangular, well impressed, not adjoining anteroventral margin of chondrophore, not extending behind chondrophore. Shell interior dull, with weak radial lines more conspicuous along ventral shell margin. Digestive tract present.

Comparisons

This species is readily distinguished from the Pacific species *S. australis, S. parkinsonii, S. pervernicosa, S. terraereginae,* and *S. velesiana* by the lack of an internal expansion of the ligament in front of the chondrophore and a radial rib bounding the anterior margin of the posterior adductor scar (Table 2). Also, *S. valvulus* is easily distinguished from *S. panamensis* (Figs. 22–30) by the lack the radial rib bounding the anterior margin of the posterior adductor scar, in having sharpened posterior extensions of the resilium and the ovate-triangular posterior adductor scar not adjoining the anteroventral margin of the chondrophore and not extending behind the chondrophore. *Solemya valvulus* is most similar to *S. pusilla, S. tagiri,* and *S. moretonensis. Solemya valvulus* differs from *S. tagiri* in having sharpened posterior extensions of the resilium, a longer chondrophore, and alimentary canal; from *S. moretonensis* and *S. pusilla* by the lack the radial rib in front of the anterior margin of the posterior adductor scar. Moreover, as compared with *S. pusilla* (Figs. 15–21), the posterior adductor scar of *S. valvulus* does not extend behind the chondrophore.

Distribution and Habitat

Solemya (*Solemya*) *valvulus* occurs from Bahía Santiago, Colima, Mexico (19°06’24”N, 104°22’28”W) (SBMNH 83479) to Monterey, California (36.7°N), including the Gulf of California. The species has been recorded from the intertidal zone to 360 m, in fine sediments (Coan et al., 2000; P. Valentich-Scott, personal communication).

Solemya (*Solemya*) *tagiri*
Okutani, Hashimoto & Miura, 2003

Solemya (*Solemya*) *tagiri* Okutani, Hashimoto & Miura, 2003: 91–96, figs. 2–5.

Type Material and Locality

Holotype (NSMT-Mo 73543) and paratypes (NSMT-Mo 73544; Japan Marina Science & Technology Center 031576-031578, 031588-031594, 053181-053191), Kagoshima Bay (31°39.55–58’N, 130°48.14–20’E), 94–98 m (Okutani et al., 2003).

Diagnosis

Shell small (to 24 mm), elongate-oval, equivalve, strongly inequilateral. Posterior and anterior shell ends gently rounded. Sculpture of weak, fine ribs and furrows, more expressed at anterior and posterior shell ends. Periostracum thin, yellowish-brown. Beaks small, not prominent. Ligament internal, thin, subtriangular, attached to chondrophore, visible externally as sunken diamond-shaped button behind beaks, without an expansion in front of chondrophore. Chondrophore weak, very short, thin, situated in front of posterior adductor scar. Internal radial rib in front of posterior adductor scar absent. Anterior adductor scar ovate-quadrate. Posterior adductor scar rounded, extending behind chondrophore. Digestive tract absent.

Comparisons

Solemya tagiri is distinguished from *S. australis, S. parkinsonii, S. pervernicosa, S. terraereginae,* and *S. velesiana* by the lack of an internal expansion of the ligament in front of the chondrophore and a radial rib bounding the anterior margin of the posterior adductor scar (Table 2). *Solemya tagiri* differs from *S. panamensis* by the lack the radial rib bounding the anterior margin of the posterior adductor scar and in having a very short chondrophore. *Solemya tagiri* is most similar to *S. pusilla, S. valvulus,* and *S. moretonensis*; but differs in having a very short chondrophore and the lack of sharpened posterior extensions of the resilium and alimentary canal. Moreover, in contrast to *S. pusilla* and *S. moretonensis*, *S. tagiri* has no radial rib in front of the anterior margin of the posterior adductor scar.

Distribution and Habitat

Solemya (*Solemya*) *tagiri* occurs in Kagoshima Bay (southern Kyushu, Japan). This species has been recorded at depths from 76 to 116 m in the area of submarine fumaroles (Okutani et al., 2003).

Subgenus *Petrasma* Dall, 1908

Type species – *Solemya borealis* Totten, 1834, by original designation.

Diagnosis

Internal ligament broad, subtriangular, sometimes with a small, irregularly shaped lateral expansion in front of chondrophore. Chondrophore bounding anterodorsal margin of posterior adductor scar, supported by a radial rib. Anterior end of posterior adductor scar impressed into inner shell surface, adjoining anteroventral margin of chondrophore.

Remarks

The subgenus *Petrasma* differs from all subgenera of the genus *Solemya* in that the ventral margin of the chondrophore bounds the anterodorsal margin of the posterior adductor scar and the anterior end of the posterior adductor scar is impressed into the inner shell surface (Figs. 2–6, Table 1). Also, this subgenus is distinguished from almost all subgenera, except the subgenus *Austrosolemya*, by the presence of a radial rib supporting the chondrophore in front of the posterior adductor scar. The subgenus *Petrasma* further differs from the subgenera *Solemyarina*, *Zesolemya*, and *Austrosolemya* either in the lack or in shape of the lateral expansion of internal ligament in front of the chondrophore.

Solemya (*Petrasma*) *panamensis* Dall, 1908
(Figs. 22–32)

Solemya (*Petrasma*) *panamensis* Dall, 1908a: 366; Bernard, 1980: 18, fig. 2; Bernard, 1983: 9.

Type Material and Locality

Holotype (USNM 110678), Panama Bay (8°44'N, 79°09'W), 54 m, "Albatross" station 2799, collected 6 March 1888 (Dall, 1908a; Bernard, 1980; P. Valentich-Scott, personal communication).

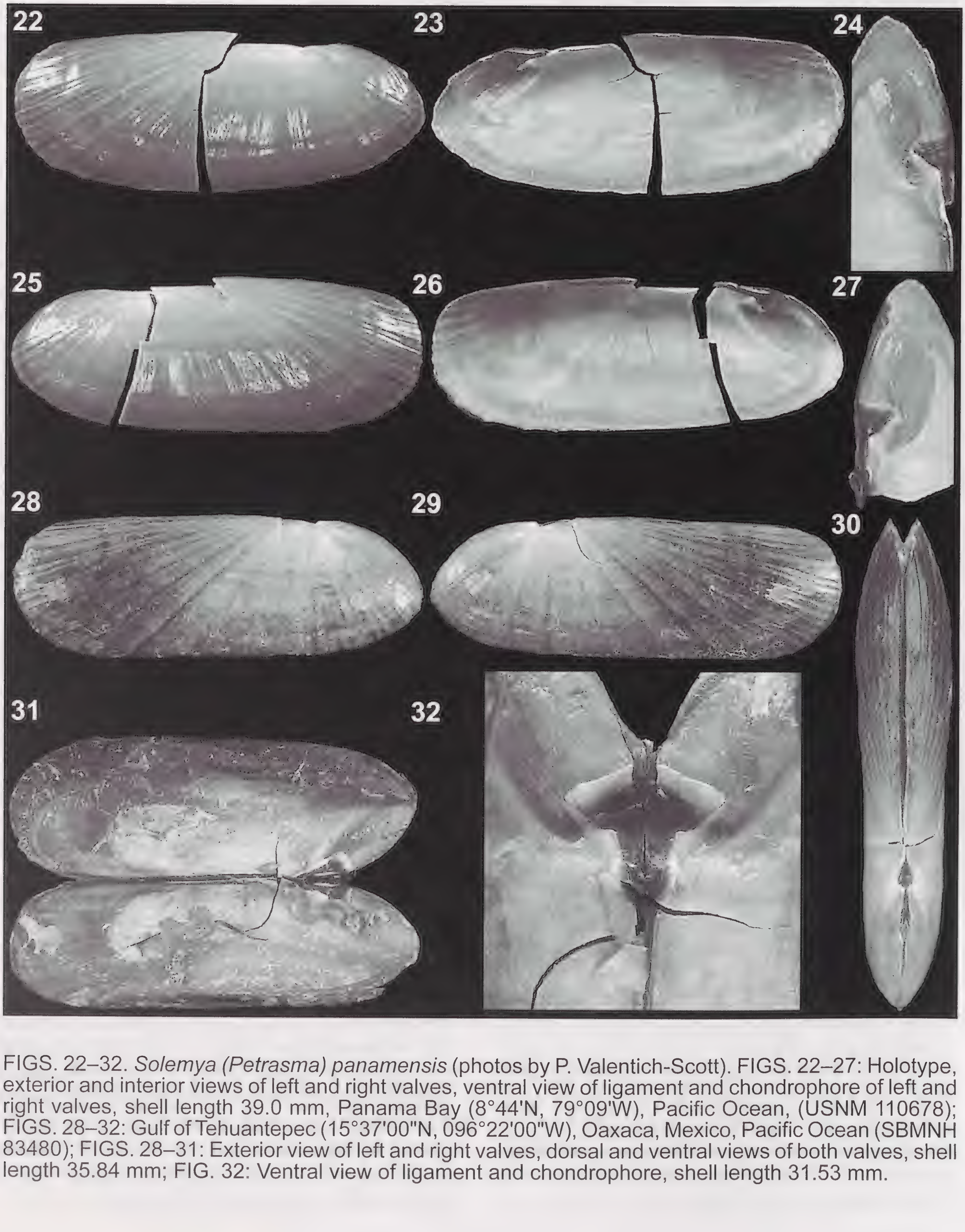
Description (expanded from Dall, 1908a)

Exterior: Shell large (to 47 mm), elongate-oval, equivalve, strongly inequilateral, thin, flexible, poorly mineralized. Sculpture of very low, broad, widely spaced radial ribs, less expressed medially and narrow, radial, thread-like ribs in space between broad ribs of anterior shell end. Periostracum thick, brown at anterior and posterior shell ends and yellowish-brown or yellow in umbonal area, widely extending beyond shell margin. Beaks very small, obscure, not prominent, near posterior end. Anterior shell end gently rounded. Posterior shell end slightly angular, pointed, with a slightly noticeable, short, radial, oblique flexure, corresponding to attachment of chondrophore to inner shell surface. Posterodorsal shell margin slightly convex in internal ligament area, forming a small, smooth, obtuse angle, smoothly transitioning to slightly pointed posterior margin. Anterodorsal shell margin straight, parallel to ventral margin, smoothly transitioning to rounded anterior margin. Ventral margin straight.

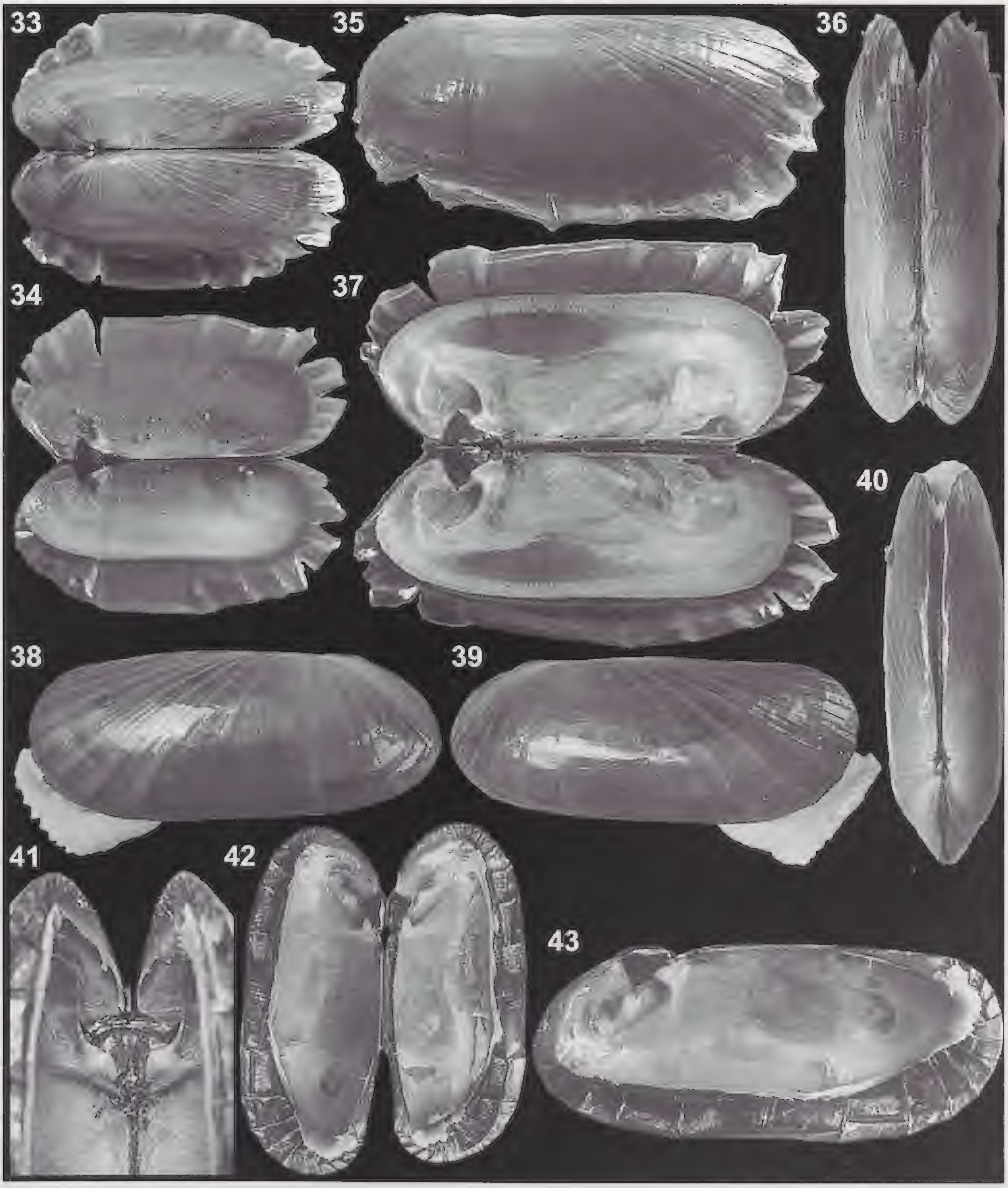
Interior: Ligament thick, subtriangular, broad, attached to chondrophore, without an expansion in front of chondrophore, visible externally as large, deep-brown, lozenge-shaped button behind beaks. Chondrophore strong, short, thin, oblique, bounding anterodorsal margin of posterior adductor scar, supported by a weak radial rib bounding anterior margin of posterior adductor scar. Anterior adductor scar large, angular-oval, feebly impressed. Posterior scar smaller, pear-shaped, well impressed, adjoining anteroventral margin of chondrophore, extending behind chondrophore; anterior end of posterior adductor scar impressed into inner shell surface, forming an arcuate cavity under chondrophore. Shell interior dull, with weak radial lines more conspicuous along ventral shell margin. Digestive tract present.

Comparisons

Solemya panamensis is most similar to *S. australis, S. pervernicosa,* and *S. parkinsonii* in the shell size, form, and proportions; but it easily differs from these species in the absence of an internal ligament expansion in front of the chondrophore (Table 2). Moreover, in contrast to *S. australis* (Figs. 88–94), this species has a thin chondrophore and a weak, narrow radial rib supporting the chondrophore. Unlike *S. parkinsonii* (Figs. 95–100), it has no posterior extensions of the resilium; its narrow internal radial rib supports the chondrophore; the an-



FIGS. 22–32. *Solemya (Petrasma) panamensis* (photos by P. Valentich-Scott). FIGS. 22–27: Holotype, exterior and interior views of left and right valves, ventral view of ligament and chondrophore of left and right valves, shell length 39.0 mm, Panama Bay (8°44'N, 79°09'W), Pacific Ocean, (USNM 110678); FIGS. 28–32: Gulf of Tehuantepec (15°37'00"N, 096°22'00"W), Oaxaca, Mexico, Pacific Ocean (SBMNH 83480); FIGS. 28–31: Exterior view of left and right valves, dorsal and ventral views of both valves, shell length 35.84 mm; FIG. 32: Ventral view of ligament and chondrophore, shell length 31.53 mm.



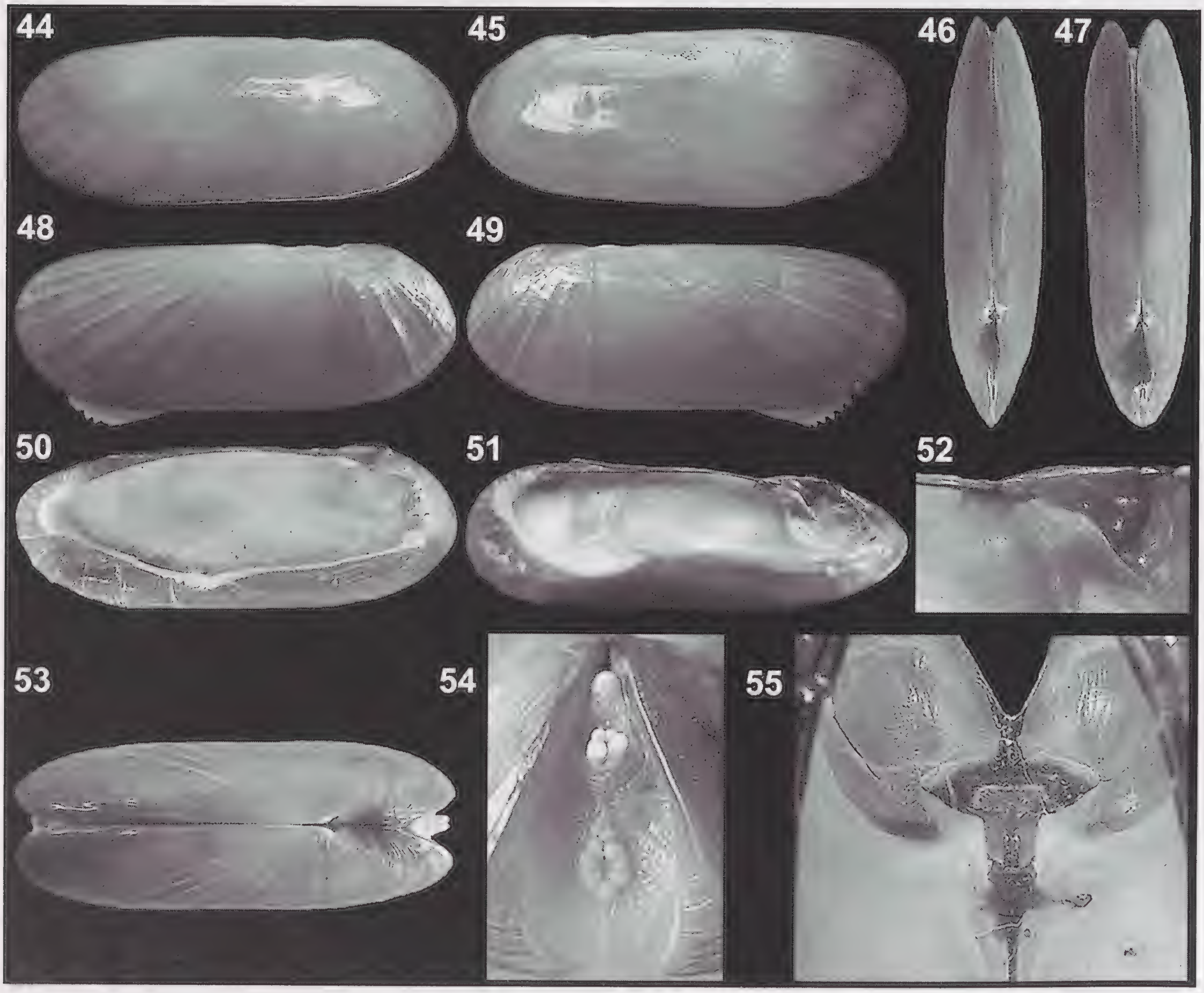
FIGS. 33–43. *Solemya (Petrasma) pervernicosa* from the northeastern Pacific. FIGS. 33, 34: Exterior and interior views of both valves, shell length 18.1 mm, Anthony Island (52°11.81'N, 131°15.10'W), Queen Charlotte Islands, British Columbia (RBCM 999-00210-012); FIGS. 35–37: Exterior view of right valve, dorsal and interior views of both valves, shell length 44.5 mm (RBCM 999-00210-012); FIGS. 38–43: Exterior view of left and right valves, dorsal view of both valves, ventral view of ligament and chondrophore, interior view of both valves and left valve, shell length 55.9 mm, Bawden Point, 75 m east (49°18.505'N, 126°00.320'W), Clayoquot Sound, Vancouver Island, British Columbia (MIMB 19964, donation by S. J. Lipovsky, CS).

terior end of the posterior adductor scar is impressed into the inner shell surface and adjoins the anteroventral margin of the chondrophore. In contrast to *S. pervernicosa* (Figs. 33–87), *S. panamensis* has a thin chondrophore and alimentary tract; internal radial rib supports the anterior end of chondrophore.

Solemya panamensis is further readily distinguished from *S. terraereginae* and *S. velesiana* by the lack of an internal ligament expansion in front of the chondrophore (Table 2). Unlike *S. pusilla, S. valvulus,* and *S. tagiri* which also have no internal ligament expansion in front of the chondrophore, *S. panamensis* lacks sharpened posterior extensions of the resilium; the chondrophore is supported by the radial rib; the anterior end of the posterior adductor scar is impressed into the inner shell surface and adjoins the anteroventral margin of the chondrophore.

Distribution and Habitat

Solemya (*Petrasma*) *panamensis* occurs from Panama Bay (8°44'N, 79°09'W) to Gulf of Tehuantepec, Oaxaca, Mexico (15°37'00"N, 096°22'00"W) (SBMNH 83480). The species has been recorded from 20 m to 235 m, on silt (Dall, 1908a; Bernard, 1983; P. Valentich-Scott, personal communication).



FIGS. 44–55. Young specimens of *Solemya (Petrasma) pervernicosa* from the northeastern Pacific. FIGS. 44–46: Exterior views of left and right valves, dorsal view of both valves, shell length 19.0 mm, Bawden Point, 75 m east (49°18.505'N, 126°00.320'W), Clayoquot Sound, Vancouver Island, British Columbia (MIMB 19964, donation by S. J. Lipovsky, CS); FIGS. 47–54: Specimens from Santa Monica Bay (33°55.70'N, 118°33.41'W), California (MIMB 19965, donation by T. Phillips, EMD); FIGS. 47–52: Dorsal view of both valves, exterior view of left and right valves, interior and ventral views of right valve, ventral view of ligament and chondrophore of right valve, shell length 22.5 mm; FIGS. 53, 54: Dorsal view of both valves, posterior aperture, shell length 24.6 mm; FIG. 55: Ventral view of ligament and chondrophore, Santa Monica Bay, California, shell length 35.0 mm (from Coan et al., 2000).

Solemya (*Petrasma*) *pervernicosa* Kuroda, 1948 (Figs. 33–87, Table 3)

Solemya (*Petrasma*) *pervernicosa* Kuroda, 1948: 32, figs. 4, 5; Kira, 1959: 194, pl. 71, fig. 3.
Petrasma pervernicosa (Kuroda, 1948) – Habe, 1977: 11; Habe, 1981: 25; Okutani et al., 1988: 21; Okutani, 2000: 833, pl. 414, fig. 4.
Petrasma pusilla (Gould, 1861) – Ito, 1985: 32, pl. 11, fig. 1.
Petrasma pervernicosa Kuroda, 1948 – Higo et al., 1999: 413.
Solemya reidi Bernard, 1980: 19–22, fig. 4; Bernard, 1983: 9; Valentich-Scott, 1998: 106–107, fig. 5.1; Coan et al., 2000: 65, pl. 1.
?*Petrasma atacama* Kuznetsov & Schileyko, 1984: 39–42, figs. 1, 2.

Type Material and Locality

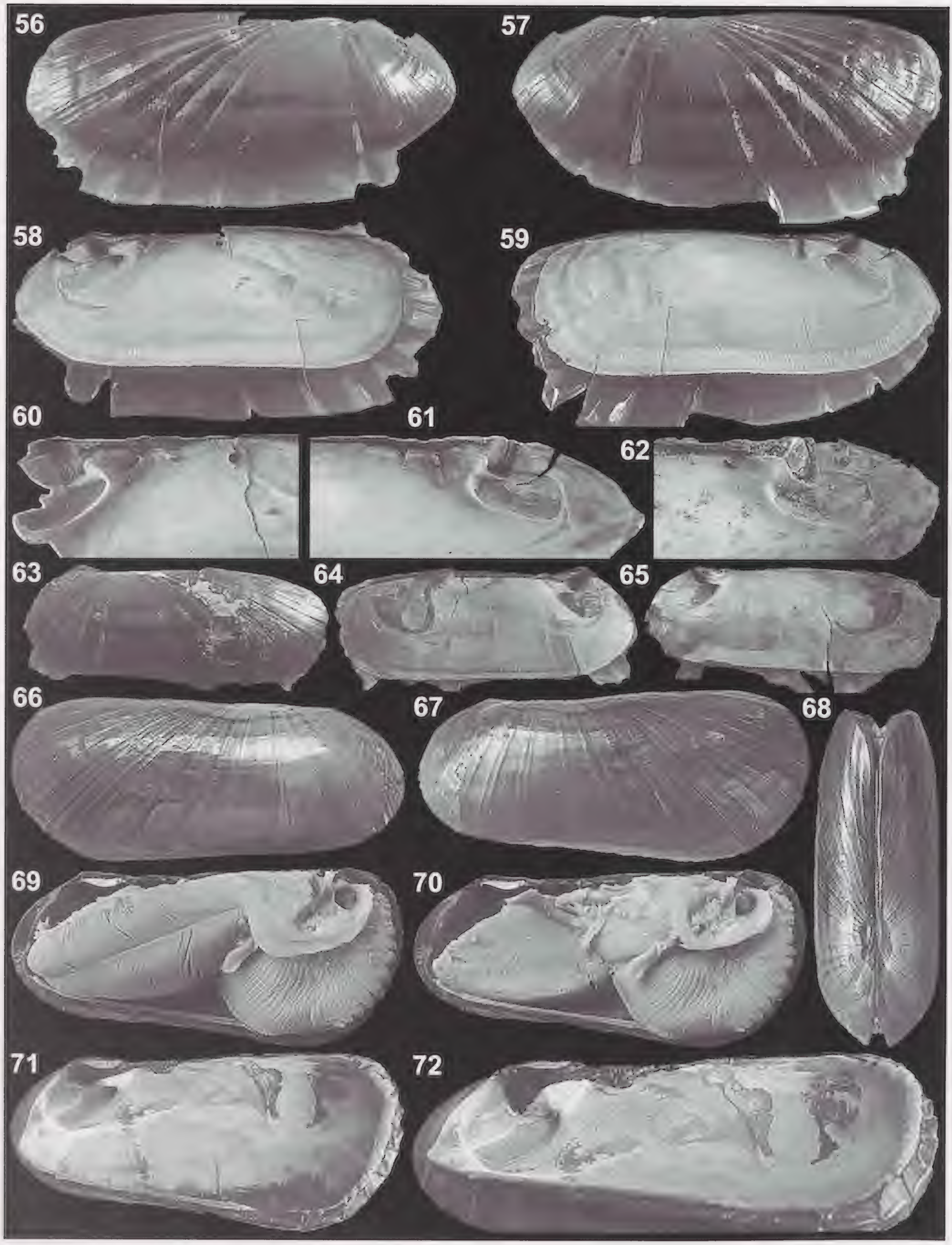
Solemya (*Petrasma*) *pervernicosa* – holotype (Nishinomiya Shell Museum, NC-H345), Cape Erimo, southeastern Hokkaido, 455 m (Kuroda, 1948; Higo et al., 1999; Hiroshi Saito (NSMT), personal communication); *Solemya reidi* – holotype (National Museum of Canada, 86074), Texada Island, northern Strait of Georgia, British Columbia, Canada (49°4'N, 124°33.2'W), 326 m (Bernard, 1980); *Petrasma atacama* – neither type or any other materials have not been found (IO RAS, ZMMU), near the Peru-Chile Trench (7°41'S, 79°47'W), 105 m (Kuznetsov & Schileyko, 1984).

Material Examined

One specimen (MIMB 19962), Sakhalin Island (46°51'N, 143°58.7'E – 47°02'N, 143°48.5'E), Sea of Okhotsk, 300–800 m, Coll. A. A. Balanov, 22.07.1997; Shell fragments of two specimens (IMB 19963), Tatar Strait, Sakhalin Island (48°20.9'N, 141°27.8'E – 48°29.2'N, 141°27.9'E), Sea of Japan, 330 m, Coll. G. M. Kamenev, 11.11.2003; 4 specimens (IO RAS), near the Peru-Chile Trench

TABLE 3. *Solemya (Petrasma) pervernicosa.* Shell measurements (mm), indices and summary statistics: L – shell length (without periostracum); H – height; A – anterior end length.

Area		L	H	A	H/L	A/L
Chiba Prefecture, Japan (NSMT Mo 76720)		60.0	21.0	39.0	0.350	0.650
Chiba Prefecture, Japan (NSMT Mo 76720)		43.0	16.0	29.0	0.372	0.674
Chiba Prefecture, Japan (NSMT Mo 76720)		50.0	18.0	35.0	0.360	0.700
Sakhalin Island, Sea of Okhotsk		63.1	24.5	41.1	0.388	0.651
Anthony Island, Queen Charlotte Islands, British Columbia (RBCM 999-00210-012)		44.5	17.0	31.0	0.382	0.697
Anthony Island, Queen Charlotte Islands, British Columbia (RBCM 999-00210-012)		21.4	8.5	15.3	0.397	0.715
Anthony Island, Queen Charlotte Islands, British Columbia (RBCM 999-00210-012)		18.1	6.2	12.6	0.343	0.696
Clayoquot Sound, Vancouver Island, British Columbia		55.9	21.8	34.2	0.390	0.612
Clayoquot Sound, Vancouver Island, British Columbia		19.0	7.5	12.1	0.395	0.637
Clayoquot Sound, Vancouver Island, British Columbia		3.8	1.6	2.7	0.421	0.711
Santa Monica Bay (33°54.45'N, 118º31.46'W), California		24.6	9.0	16.1	0.365	0.654
Santa Monica Bay (33°54.45'N, 118º31.46'W), California		22.5	8.4	14.8	0.373	0.658
Santa Monica Bay (33°55.70'N, 118º33.41'W), California		16.3	5.8	10.8	0.356	0.663
	Mean	-	-	-	0.376	0.671
	SE	-	-	-	0.006	0.009
	SD	-	-	-	0.022	0.031
	Min	3.8	1.6	2.7	0.343	0.612
	Max	63.1	24.5	41.1	0.421	0.715
	N	13	13	13	13	13



FIGS. 56–72. *Solemya (Petrasma) pervernicosa* from the northwestern Pacific. FIGS. 56–65: Specimens from Loc. Choshi, Chiba Prefecture, Japan (NSMT Mo 76720); FIGS. 56–61: Exterior and interior views and ventral view of ligament and chondrophore of left and right valves, shell length 58.0 mm; FIG. 62: Ventral view of ligament and chondrophore of right valve, shell length 50.0 mm; FIGS. 63–65: Exterior view of right valve, interior view of right and left valves, shell length 43.0 mm; FIGS. 66–72: Specimen from Sakhalin Island (46°51'N, 143°58.7'E – 47°02'N, 143°48.5'E), Sea of Okhotsk (MIMB 19962), exterior views of left and right valves, dorsal view of both valves, right side after removal of right valve and mantle, right side after removal of right valve, mantle, and gill, interior and ventral views of left valve, shell length 63.1 mm.

(5°57'S, 81°15.7'W), Pacific Ocean, 330 m, clayey silt, R/V "Akademik Kurchatov"(cruise 4), 4.11.1968; *P. pervernicosa* – 3 specimens (NSMT Mo 76720), Loc. Choshi, Chiba Prefecture, Japan; *S. reidi* – 3 specimens (RBCM 999-00210-012), Anthony Island (52°11.81'N, 131°15.10'W), Queen Charlotte Islands, British Columbia, Canada, 63.5 m, Coll. P. Lambert, 17.06.1999; 1 specimen (RBCM 001-00126-002), Montagu Channel, Canada, 14.02.1977; 3 specimens (MIMB 19964, donation by S. J. Lipovsky, CS), Bawden Point, 75 m east (49°18.505'N, 126°00.320'W), Clayoquot Sound, Vancouver Island, British Columbia, Canada, 101 m, Coll. B. Taekema, 30.05.2006; 2 specimens (MIMB 19965, donation by T. Phillips, EMD), Santa Monica Bay (33°54.45'N, 118°31.46'W), California, 60 m, Coll. T. Phillips, 31.07.2006; 1 specimen (MIMB 19966, donation by T. Phillips, EMD), Santa Monica Bay (33°55.70'N, 118°33.41'W), California, 156 m, Coll. T. Phillips, 10.07.2006. Total of 18 specimens and shell fragments of 2 specimens.

Description (expanded from Bernard, 1980)

Exterior: Shell large (to 65 mm), elongate-oval (H/L = 0.343–0.421), moderately inflated, equivalve, strongly inequilateral, thin, flexible, poorly mineralized (dry shell very fragile), white under periostracum, gaping anteriorly. Sculpture of very low, broad, widely spaced radial ribs, less expressed and often absent medially, and narrow, radial, thread-like ribs in space between broad ribs of anterior shell end. Periostracum thick, dark chestnut-brown, with a black oval or elongate-oval spot in umbonal area, widely extending beyond shell margin. Beaks very small, obscure, not prominent, near posterior end (A/L = 0.612–0.715), situated in front of black, uncalcified area of shell covering a lateral expansion of internal ligament. Posterior shell end rounded, slightly angular. Anterior end broadly rounded. Posterodorsal shell margin slightly convex in internal ligament area, forming a small, smooth, obtuse angle, smoothly transitioning to slightly angular posterior margin. Anterodorsal shell margin straight, parallel to ventral margin, smoothly transitioning to rounded anterior margin. Ventral margin straight.

Interior: Ligament thick, strong, subtriangular, attached to chondrophore, with an oval or elongate-oval expansion (often irregular in form and extending along dorsal margin) in front of chondrophore. Chondrophore very strong, short, oblique, bounding anterodorsal margin of posterior adductor scar, supported by a weak, low, radial rib bounding anterior margin of posterior adductor scar. Anterior adductor scar large, angular-oval, feebly impressed. Posterior scar smaller, pear-shaped, well impressed, adjoining anteroventral margin of chondrophore, extending behind chondrophore; anterior end of posterior adductor scar impressed into inner shell surface and adjoining anteroventral margin of chondrophore forming an arcuate cavity under chondrophore. Shell interior dull, with weak radial lines more conspicuous along ventral shell margin. Posterior aperture of young specimens with a large, dorsal papilla, two pairs of suprasiphonal papillae (ventral pair longer and thicker), and 12 siphonal papillae increasing in size ventrally. Digestive tract absent.

Variability

Shell shape and proportions vary slightly (Figs. 33–87, Table 3). The width and number of radial ribs, especially in the posterior shell end, are also slightly variable. In young specimens, the radial ribs in the anterior and posterior ends of shell are more noticeable (Figs. 33, 44–49, 77, 78), the color of the periostracum is markedly lighter, bright brown, sometimes with a reddish hue or more uniform. Form of the external, black uncalcified spot in the umbonal area markedly varies. In young specimens, this spot is distinctly diamond-shaped or triangular and is situated just behind the beaks (Figs. 46, 47, 53, 79, 85). In adults, the relative size and form of this spot greatly vary from weakly visible, small, rounded to large, bright black, elongate-oval (Figs. 36, 40, 68). Correspondingly, highly variable are the relative size, width, and form of the internal expansion of the ligament in front of the chondrophore (Figs. 37, 41, 55, 60–62, 72). The posterior scar in adults varies from rounded-elongate, pear-shaped (Figs. 43, 58, 59, 71) to angular, trigonal-rounded, with an acute angle between the dorsal and anterior margins of the scar (Fig. 37).

Distribution and Habitat

Solemya (*Solemya*) *pervernicosa* occurs in the western Pacific off Japan from the Kii Peninsula (Honshu, Pacific Ocean) to Cape Erimo (Hokkaido, Pacific Ocean) (Habe, 1981; Okutani et al., 1988; Higo et al., 1999; Okutani, 2000), off Kuriya, Awa Island (Sea of Japan) (Ito, 1985; Okutani et al., 1988), off western Sakhalin Island (Tatar Strait, Sea of Japan), off southeastern Sakhalin Island (Sea of Okhotsk); in the eastern Pacific from southeast Alaska (58°N) to Peru (7°41'S) (Bernard, 1980, 1983; Kuznetsov & Schileyko, 1984; Valentich-Scott, 1998; Coan et al., 2000). This species has been recorded at depths from 40 to 1510 m (Sagami Bay, Japan) in areas of high organic content and low oxygen levels (Okutani et al., 1988; Valentich-Scott, 1998; Coan et al., 2000; Okutani, 2000).

Comparisons

Solemya pervernicosa is readily distinguished from the Pacific species *S. valvulus*, *S. pusilla*, and *S. tagiri*, by the presence of an internal expansion of the ligament in front of the chondrophore and a radial rib supporting the chondrophore and bounding the anterior margin of the posterior adductor scar (Table 2). Likewise, it readily differs from *S. terraereginae, S. velesiana,* and *S. moretonensis* in the shapes of the resilium and chondrophore, and the ligament expansion in front of chondrophore.

Solemya pervernicosa is most similar to *S. australis, S. panamensis,* and *S. parkinsonii* in the shell size, form, and proportions; but it easily differs from these species in the shape of the internal ligament expansion, the position and appearance of the radial rib supporting the chondrophore, as well as the shape and position of the chondrophore and the posterior adductor scar.

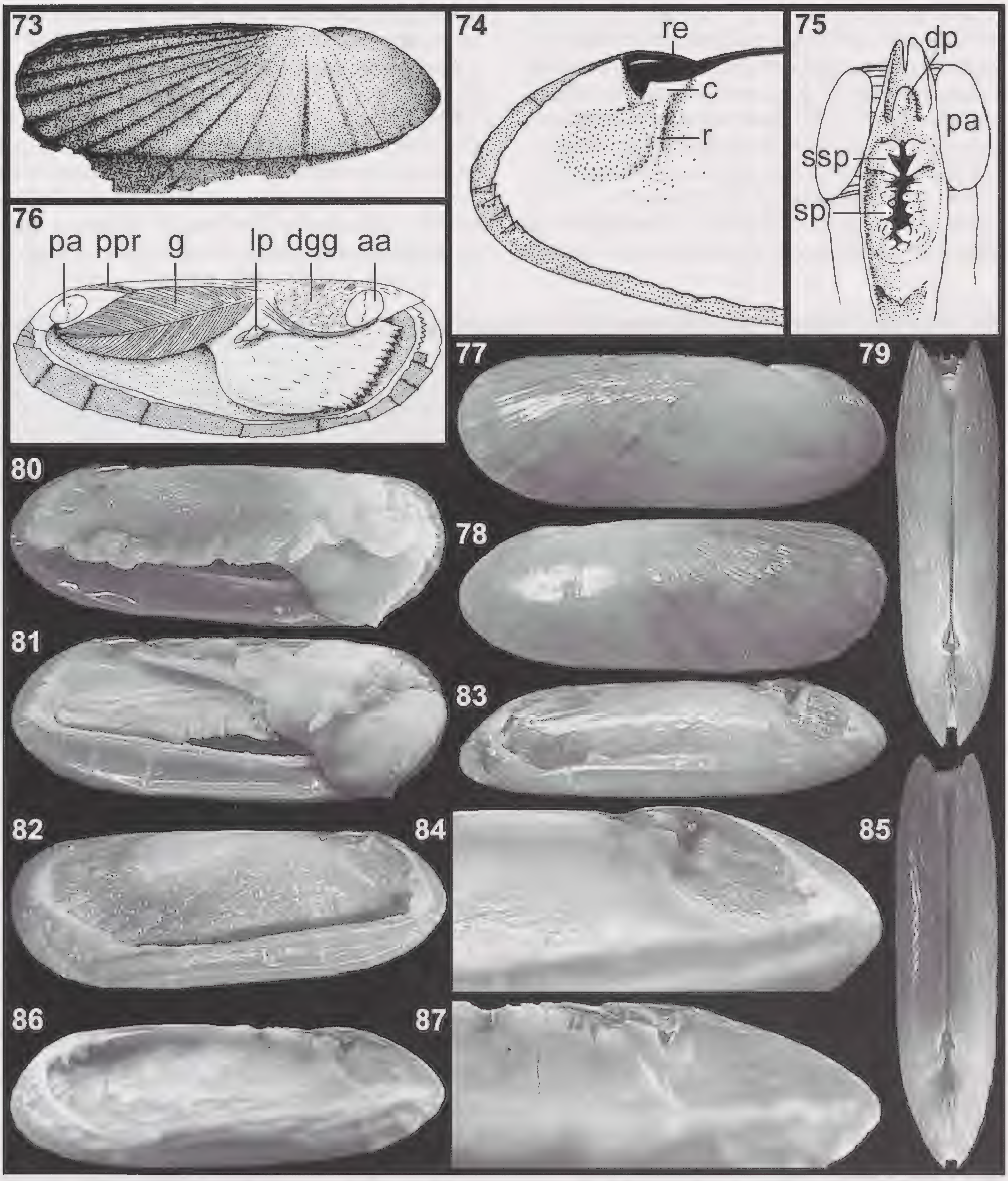
Solemya pervernicosa primarily differs from *S. panamensis* in the presence of an expansion of the ligament in front of the chondrophore, the lack of alimentary canal, and in having a stronger and thicker chondrophore with a radial rib supporting the posterior, but not the anterior end of the chondrophore (Figs. 23, 26, 37, 43, 58, 59, 71).

In *S. australis* (Figs. 88–94), unlike *S. pervernicosa*, the internal ligament expansion looks like two large oval lobes that are positioned in a shallow depression on the inner shell surface. To these lobes corresponds a short, black or gray stria on the outer surface of the shell in the umbonal area that crosses the dorsal shell margin at a straight angle. The interior radial rib that supports the chondrophore is strong, well expressed, broad, projecting above the inner shell surface as a ridge in front of the posterior adductor scar. In contrast to *S. pervernicosa*, this rib supports the anterior, but not the posterior end of the chondrophore, and touches the internal lobes of the ligament. Moreover, the posterior adductor scar is not impressed into the inner shell surface; the chondrophore is short and narrowed posteriorly.

In *S. parkinsonii* (Figs. 95–100), in comparison with *S. pervernicosa*, the internal expansion of the ligament extends laterally as well-marked, narrow, long striae, which are also visible on the external shell surface. These striae of the ligament are situated in the corresponding furrows on the inner surface of the shell. The chondrophore is long, thin, thickening posteriorly, and not supported by a radial rib. Weakly expressed radial ribs on the inner shell surface appear as broad, low, rounded ridges extending ventrally from the dorsal shell margin between the chondrophore and lateral striae of the ligament. The posterior adductor scar is small, rounded-triangular, not adjoining the chondrophore.

Remarks

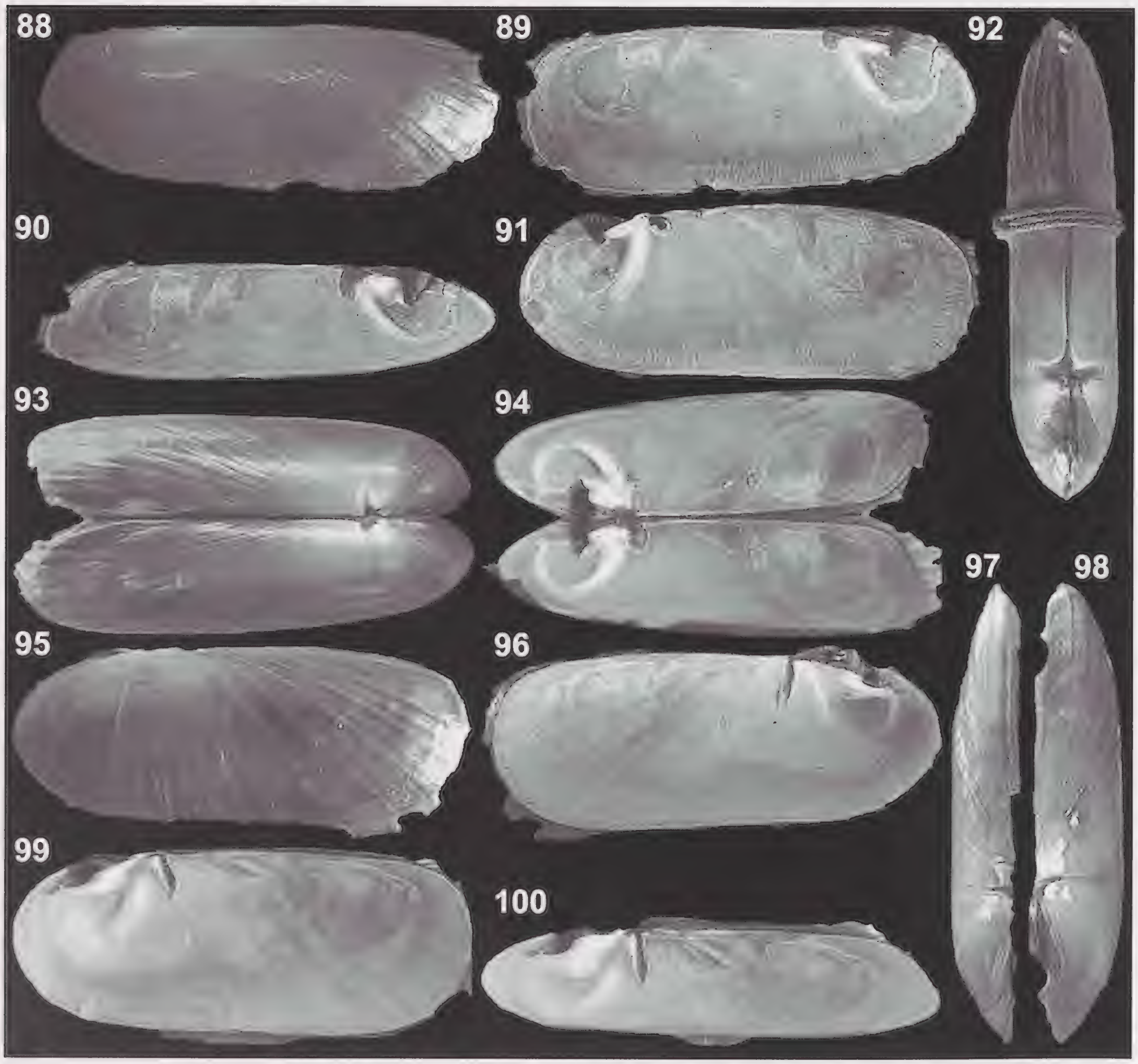
The study of *Solemya* species from the Pacific seas of Russia, as well as *S. reidi* from the northeastern Pacific showed that these species are identical to *S. pervernicosa*. In the description of *S. reidi*, Bernard (1980) made a detailed comparison of this species with other species of the genus *Solemya*. Unfortunately, he did not compare *S. reidi* with the large, well-identifiable *S. pervernicosa* found off Japan. Indeed, these species are similar in size, shape, proportions, and external and internal shell morphology (Figs. 33–43, 56–72, Table 3). Both species have a dark chestnut-brown periostracum, an identical shell sculpture of broad, low, widely spaced radial ribs and very fine radial ribs in the space between broad ribs, and a strong, subtriangular internal ligament attached to oblique, strong chondrophore. The chondrophore is supported by a radial rib bounding the anterior margin of the posterior adductor scar. This rib is weakly expressed and is mostly formed by the deeply impressed posterior adductor scar. The form and arrangement of the anterior and posterior adductor scars, taking into account their individual variability, are also identical. The two species have an internal expansion of the ligament in front of the chondrophore. The form of this expansion varies markedly. In adult specimens of *S. reidi* from British Columbia and California, this ligament expansion appears as well noticeable, fairly thick, rounded lateral patches, or it is oval with short lateral rays of varying length (Figs. 37, 41). In young specimens of *S. reidi*, the form of this expansion is also variable (Figs. 34, 51, 52, 55). A specimen from the southern Sea of Okhotsk has a thick,



FIGS. 73–87. *Solemya (Petrasma) pervernicosa* from the southeastern Pacific. FIGS. 73–76: *Petrasma atacama*, holotype, exterior view of left valve, interior view of ligament and chondrophore of left valve, posterior aperture, right side after removal of right valve and mantle, shell length 14.0 mm, Peru-Chile Trench (5°57'S, 81°15.7'W) (from Kuznetsov & Schileyko, 1984, figs. 1A, 2A, B, C); FIGS. 77–87: Peru-Chile Trench (5°57'S, 81°15.7'W), Pacific Ocean (IO RAS). FIGS. 77–84: External view of left and right valves, dorsal view of both valves, right side after removal of right valve and mantle, right side after removal of right valve, mantle, and gill, interior and ventral views of right valve, ventral view of ligament and chondrophore of right valve, shell length 25.0 mm; FIG. 85: Dorsal view of both valves, shell length 23.7 mm; FIGS. 86–87: Ventral view of right valve, ventral view of ligament and chondrophore of right valve, shell length 19.7 mm. aa, anterior adductor muscle; c, chondrophore; dgg, digestive gland and gonad; dp, dorsal papilla; g, gill; lp, labial palp; pa, posterior adductor muscle; ppr, posterior pedal retractor muscle; r, radial rib in front of posterior adductor scar; re, resilium; sp, siphonal papillae; ssp, suprasiphonal papillae.

knob-like, oval expansion of the ligament (Figs. 71, 72). In *S. pervernicosa* from the Pacific waters of Honshu, Japan, the internal expansion of the ligament is less expressed, thin, elongate, extending along the dorsal margin, sometimes with sparse, short lateral rays (Figs. 60–62). My opinion is that this difference in the form of the internal ligament expansion in front of the chondrophore, taking into account its individual variability, is not a specific characteristic. Therefore, I consider *S. reidi* to be a synonym of *S. pervernicosa*.

Kuznetsov & Schileyko (1984) described *Petrasma atacama* Kusnetzov & Schileyko, 1984, on the basis of material from the eastern Pacific (Figs. 73–76). Unfortunately, my attempts to find any original material of this species in the collections of IO RAS and ZMMU were unsuccessful. *Petrasma atacama* was described by Kuznetsov & Schileyko (1984)



FIGS. 88–100. *Solemya (Austrosolemya) australis* and *Solemya (Zesolemya) parkinsonii*. FIGS. 88–94: *Solemya (Austrosolemya) australis*, Spencer Gulf (34°0.000'S, 137°0.000'E), South Australia (AMS C 458812); FIGS. 88–90: External, internal, and ventral views of right valve, shell length 50.0 mm; FIG. 91: Internal view of left valve, shell length 44.0 mm; FIGS. 92–94: *Solemya (Austrosolemya) australis*, 35°15.000'S, 137°0.000'E, South Australia (AMS C 085088); FIG. 92: Dorsal view of both valves, shell length 38.0 mm; FIGS. 93, 94: External and internal views of both valves, shell length 45.0 mm; FIGS. 95–100: *Solemya (Zesolemya) parkinsonii*, external and internal views of right valve, dorsal view of left and right valves, internal and ventral views of left valve, shell length 35.5 mm, N Douglas, Auckland, Tamaki Strait, Kawaka Bay, North Island, New Zealand (NMNZ M 274993).

from nine specimens. The authors designated a holotype and three paratypes, provided shell measurements of the holotype (the largest specimen: L = 14.0 mm, H = 5.7 mm, W = 3.0 mm), and described the shell morphology and anatomy of this species. However, there was no indication whether the lateral expansion of the ligament and the corresponding uncalcified area of the shell in the umbonal area are present or lacking. Since it was assigned to the genus *Petrasma*, this species presumably had no internal lateral expansion of the ligament. *Petrasma atacama* was only compared by the authors with *S. valvulus*, from which it primarily differed in the presence of the radial rib supporting the chondrophore and the lack of alimentary canal. Because of the insufficiently complete description and comparative analysis of this species, a number of authors repeatedly noted that *P. atacama* requires a more detailed study and comparison with the closest species *S. reidi* and *S. panamensis* (Valentich-Scott, 1998; Coan et al., 2000). Judging from its description, *S. atacama* is identical in shell morphology to *S. panamensis* (Figs. 22, 25, 28, 29, 73); however, it differs from the latter species in the lack of alimentary canal (Bernard, 1980; Kuznetsov & Schileyko, 1984). This species is also close to *S. pervernicosa.* They have the same structure of the chondrophore and similar features of the posterior aperture (Figs. 54, 75) and lack the alimentary canal. However, *P. atacama*, unlike *S. pervernicosa,* have no lateral expansion of the ligament in front of the chondrophore. *Petrasma atacama* was represented by small specimens, in which this feature is usually weakly expressed, as, for example, in the investigated young specimens of *S. pervernicosa* with a shell length of 16 to 25 mm (Figs. 34, 51, 52). It is possible that the authors describing this species paid no attention to this poorly visible feature. Therefore, I think it is likely that *P. atacama* was described from the young specimens of *S. pervernicosa.*

A revision of the entire bivalve collection of the IO RAS revealed four specimens of a species of *Solemya*, with a shell length of 19.4–25.0 mm (Figs. 77–87), which were collected near the Peru-Chile Trench (5°57'S, 81°15.7'W), at the 330 m depth, relatively near the area where the *P. atacama* material was sampled.

These specimens of *Solemya* species may relate to *P. atacama* because they are very similar in proportions and the external morphology of the shell. Furthermore, this *Solemya* species also lacks an alimentary canal. However, in contrast to *P. atacama*, it has the internal expansion of the ligament in front of the chondrophore (Figs. 84, 87). This species is most similar to *S. pervernicosa*. Unlike the similar-sized specimens of *S. pervernicosa*, it has a more elongate shell (H/L = 0.304–0.350), a more pointed posterior end, finer posterior ribs, and a thinner chondrophore (Figs. 77, 78, 82–84). Possibly, these differences are due to the individual variability of *S. pervernicosa*, therefore, I assume that this *Solemya* species is probably *S. pervernicosa.*

It is well known that the different species of the genus *Solemya* harbor symbiotic sulfur-oxidizing chemoautotrophic bacteria, on which they depend to varying degrees. This leads to the reduction or loss of the gut (Reid, 1980; Doeller, 1984; Kuznetsov & Schileyko, 1984; Doeller et al., 1988; Kuznetsov et al., 1990; Krueger et al., 1996a, b; Okutani et al., 2003). Solemyid bivalves thrive in sediments with high loads of organic matter, hydrogen sulfide, and low concentration of oxygen. They were also found at methane cold seeps and in sediments influenced by hydrothermal venting (Sibuet & Olu, 1998; Okutani et al., 2003).

Solemya pervernicosa is a typical chemoautotrophic species, which is highly specialized for chemoautotrophic nutrition. It has endosymbiotic chemoautotrophic bacteria and completely lacks an alimentary canal (Bernard, 1980; Reid, 1980; Felbeck, 1983; Fisher & Childress, 1984, 1986; Powell & Somero, 1985; Anderson et al., 1987, 1990; Lee et al., 1992; Wilmot & Vetter, 1992). In the northeastern Pacific, this species is frequently found in areas of sewage outfalls or with abundant woody debris, which are highly enriched in organics, low in oxygen, and high in hydrogen sulfide (Reid, 1980; Valentich-Scott, 1998; Coan et al., 2000). In the northwestern Pacific, it was recorded in cold seeps areas. Off Japan, this species was found at the 1,510 m depth in Sagami Bay (Okutani et al., 1988; Okutani, 2000), where deep water is characterized by the abundance of methane seeps supporting dense aggregations of various chemoautotrophic bivalves (Okutani, 1957, 1962; Okutani & Egawa, 1985; Kojima & Ohta, 1997; Kamenev et al., 2001). In the Sea of Okhotsk, *S. pervernicosa* was found on the southeastern Sakhalin slope, where methane seeps also exist (Obzhirov, 1993). A chemoautotrophic thyasirid species, *Conchocele bisecta* (Conrad, 1849), was earlier found in almost the same area (Kamenev et al., 2001). A large number of active seeps of free methane or methane-rich fluids with gas hydrates have been reported to exist in the bottom sediments of the eastern Sakhalin slope and on the sea floor of the Derugin Basin (Obzhirov, 1993; Biebow & Hütten, 1999; Greinert et al., 2002; Sahling et al., 2003; Obzhirov et al., 2007). Numerous chemoautotrophic bivalves *Calyptogena rectimargo* Scarlato, 1981, *C. bisecta*, *Acharax* sp., pogonophoran tube worms, and bacterial mats are associated with methane seeps areas off eastern Sakhalin (Kamenev, 2001; Sahling et al., 2003; Krylova & Sahling, 2006). Cold-water methane-rich seep zones are fairly widely distributed in the Sea of Okhotsk (Avdeiko et al., 1984; Obzhirov, 1993; Gaedicke et al., 1997; Obzhirov et al., 2007). It is therefore quite likely that *S. pervernicosa* will be found in many of them.

Genus *Acharax* Dall, 1908

Type species – *Solemya johnsoni* Dall, 1891, by original designation.

Diagnosis

Shell moderately thick, poorly mineralized, elongate-oval, compressed, inequilateral, equivalve. Beaks obscure, not prominent, strongly posterior. Periostracum thick, adherent, varnished, extending beyond shell. Surface with widely spaced radial ribs. Lunule and escutcheon absent. Ligament opisthodetic, external, seated on a narrow nymph. Chondrophore absent. Hinge plate weak, edentate. Adductor scars unequal; posterior scar small and impressed; anterior scar large.

The genus contains 11 living species.

Acharax johnsoni (Dall, 1891)
(Figs. 101–108)

Solemya johnsoni Dall, 1891: 189.
Acharax johnsoni Dall, 1891 – Kafanov & Lutaenko, 1997: 156, 157, fig. 1, synonymy.
Acharax johnsoni (Dall, 1891) – Coan et al., 2000: 66, pl. 1, synonymy.

Type Material and Locality

Holotype (United States National Museum, No. 106886), Baja California Norte, 1,840 m, Holocene, "Albatross" (Kafanov & Lutaenko, 1997).

Material Examined

Two specimens (IO RAS), Sakhalin Island (54°28'N, 145°21'E), Sea of Okhotsk, 1,451 m, RV "Vityaz" (cruise 2, station 103), 13.09.1949; Shell fragments of one specimen (IO RAS), Sakhalin Island (54°00.5'N, 146°17'E), Sea of Okhotsk, 1,515–1,486 m, RV "Akademik Lavrentyev", Coll. S. V. Galkin, 20.08.1998; 3 specimens (IO RAS), Monterey Canyon (36°35.4'N, 122°30.5'W), Pacific Ocean, 3,040 m, RV "Akademik Mstislav Keldysh" (cruise 22, underwater vehicle "Mir-1", diving 17/61), Coll. L. I. Moskalev, 21-22.09.1990; 1 specimen (IO RAS), Peru-Chile Trench (2°00.0'S, 81°31'W), Pacific Ocean, 3,840–3,200 m, RV "Akademik Kurchatov" (cruise 4, station 305), Coll. L. I. Moskalev, 07.11.1968; Shell fragments of one specimen (IO RAS), Peru-Chile Trench (8°29.3'S, 80°46.1'W), Pacific Ocean, 4,200 m, RV "Akademik Kurchatov" (cruise 4, station 292), Coll. L. I. Moskalev, 30-31.10.1968; Shell fragments of one specimen (IO RAS), Peru-Chile Trench (12°27.8'S, 78°34.9'W), Pacific Ocean, 4,200 m, RV "Akademik Kurchatov" (cruise 4, station 277), Coll. L. I. Moskalev, 25.10.1968. Total of 6 specimens and shell fragments of 3 specimens.

Diagnosis

Shell large (to 150 mm), elongate-oval, moderately inflated, equivalve, strongly inequilateral, thick, friable. Sculpture of low, broad, widely spaced radial ribs. Periostracum thick, deep-brown to black, widely extending beyond shell margin. Beaks small, not prominent, strongly posterior. Posterior shell end rounded, narrowed. Anterior shell end truncated. Ligament opisthodetic, external, thick, attached to a nymph. Anterior adductor scar large, feebly impressed. Posterior scar smaller, well impressed. Shell interior dull, with noticeable radial ribs at anterior end, corresponding to external shell sculpture.

Remarks

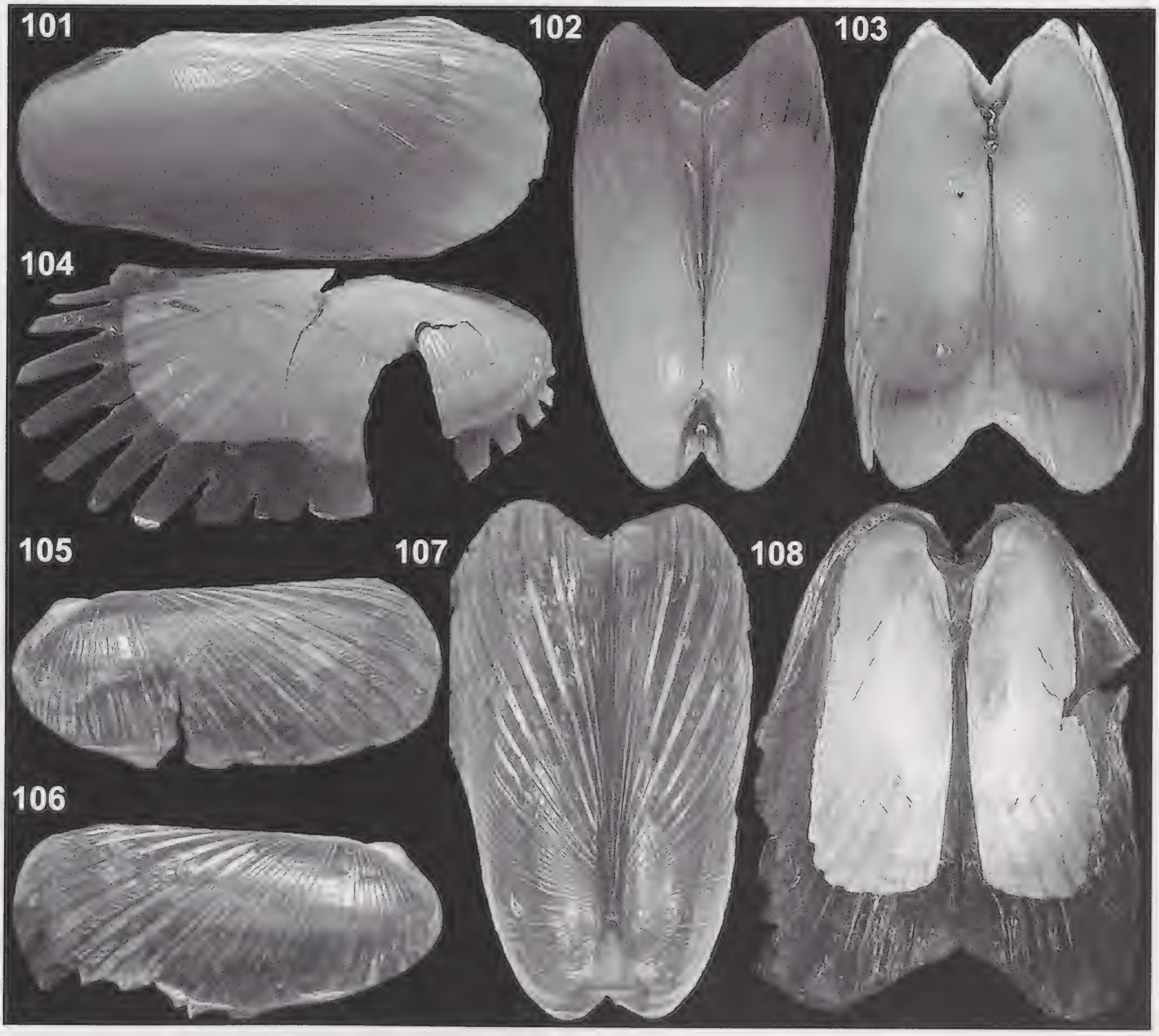
Shell morphology of all the investigated specimens and fragments of *Acharax* from the collection of the IO RAS corresponds to the diagnosis of *A. johnsoni*. Therefore, I think that all this material can be referred to *A. johnsoni.*

Until recently, it has been believed that only one species *A. johnsoni* occurs in the eastern Pacific from Alaska to Peru (Coan et al., 2000). However, genetic studies of the *Acharax* material from different areas of the Pacific and Indian Oceans showed that in the eastern Pacific there are most probably two distinct species, which, despite their morphological similarity, well separate genetically (Neulinger et al., 2006).

At present, six species of *Acharax* are known in the Pacific Ocean: *A. johnsoni; A. japonica; Acharax bartschii* Dall, 1908; *Acharax alinae* Métivier & von Cosel, 1993; *Acharax clarificata* Dell, 1995; and *Acharax patagonica* (Smith, 1885) (Dall, 1908b; Métivier & von Cosel, 1993; Dell, 1995; Coan et al., 2000; Okutani, 2000; Osorio & Reid, 2004). Most of these species are very similar in shell morphology. It is therefore no wonder that Neulinger et al. (2006), when studying the *Acharax* material from the vast region of the eastern Pacific ranging from the Aleutian Islands to Peru, revealed two species that were previously identified as one, *A. johnsoni.* The genetically studied material of *Acharax* is now deposited in the Santa Barbara Museum of Natural History (USA) (Neulinger et al., 2006; P. Valentich-Scott, personal communication). It is possible that a more thorough examination of this material will help to separate these species on the basis of shell morphology or anatomy.

Distribution and Habitat in the Pacific Ocean

Acharax johnsoni occurs in the eastern Pacific from Sitka, Alaska (57.1°N) (Coan et al., 2000) to Lima, Peru, (Peru-Chile Trench, 12°27.8'S, 78°34.9'W); in western Pacific from



FIGS. 101–108. *Acharax johnsoni.* FIGS. 101–104: Sakhalin Island (54°28'N, 145°21'E), Sea of Okhotsk, 1451 m (IO RAS); FIGS. 101–103: Exterior view of right valve, dorsal and ventral views of both valves of young specimen, shell length 19.0 mm; FIG. 104: Exterior view of left valve, shell length 43.0 mm; FIGS. 105–108: Monterey Canyon (36°35.4'N, 122°30.5'W), Pacific Ocean, 3,040 m (IO RAS), exterior view of right and left valve, dorsal and ventral views of both valves, shell length 108.0 mm.

Cape Olyutorskiy, Bering Sea (60°N) (Kafanov & Lutaenko, 1997) and northeastern Sakhalin Island, Sea of Okhotsk (54°28'N, 145°21'E) to Tosa Bay, Honshu, Japan (Higo et al., 1999). This species was recorded at depths from 100 to 5,379 m (Japan Trench, 39°06.47'N, 143°53.49'E) (Habe, 1977; Okutani, 2000; Fujikura et al., 2002; Okutani & Fujikura, 2002), on silt and clayey silt often in areas of methane seeps (Fujikura et al., 2002; Okutani & Fujikura, 2002; Fujiwara, 2003; Neulinger et al., 2006).

Remarks

In the eastern Pacific, one shell fragment of a large specimen of *Acharax* sp. was found much farther south of Lima, in the deep-sea methane seeps area off Conception, Chile (depth 934 m, 36°21.46'S, 73°44.08'W) (Sellanes et al., 2004). This shell fragment may be *A. johnsoni*. Hence, it can be assumed that in the eastern Pacific this species occurs farther south of the area of Peru-Chile Trench off Lima, Peru.

Like the species of *Solemya, A. johnsoni* harbors endosymbiotic chemoautotrophic bacteria in its gills (Fujiwara, 2003; Imhoff et al., 2003; Neulinger et al., 2006). Therefore, this species is often encountered in areas of cold methane-rich fluids and methane seeps (Fujikura et al., 2002; Okutani & Fujikura, 2002; Fujiwara, 2003; Imhoff et al., 2003; Sellanes et al., 2004; Neulinger et al., 2006). In Monterey Canyon, it was recorded in cold gas seeps areas, on a silty bottom dominated by the bivalve *Ectenagena extenta* Krylova & Moskalev, 1996 (Krylova & Moskalev, 1996). In the Sea of Okhotsk, *A. johnsoni* was found off northeastern Sakhalin Island at depths from 370 to 1,515 m in areas of methane-rich fluids and methane seepage, which support a rich fauna of chemoautotrophic species of bivalve mollusks and pogonophoran tubeworms (Kamenev et al., 2001; Sahling et al., 2003).

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TEMPERATURE AND WATER AVAILABILITY AFFECT DECREASE OF COLD HARDINESS IN THE APPLE SNAIL, *POMACEA CANALICULATA*

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ABSTRACT

The apple snail *Pomacea canaliculata* is a freshwater snail originating in tropical and temperate South America. We investigated the effect of temperature acclimatization, moisture levels, and food availability on the loss of cold hardiness in overwintering snails. Cold hardiness broke down by four days in snails maintained in aquatic condition at 25°C and by eight days at 20°C. However, snails held at 15°C retained their cold hardiness even after 64 days. In addition, the cold hardiness of snails kept moist (wrapped in wet towel) was maintained for at least 64 days even at 25°C. These results indicate that warm temperature together with increase of water availability decreases the cold hardiness of overwintering snails. Glycerol content reduced as cold hardiness decreased; supporting a previous hypothesis that glycerol has a major physiological role in the development of cold hardiness in this species.

Key words: Ampullariidae, invasive species, deacclimatization, glycerol, glucose.

INTRODUCTION

Cold hardiness, "the ability of an organism to survive at low temperature" (Leather et al., 1993), is a key trait for many ectotherms in temperate and cold region to survive cold seasons (Block, 1982; Lee & Denlinger, 1991; Ansart & Vernon, 2003). Ecological, behavioral and morphological strategies to cold seasons are known in invertebrates (Lencioni, 2004; Block, 1982), and physiological change (e.g., increase of sugars, antifreeze proteins and polyols) to increase their cold hardiness is well studied especially in insects (Lee & Denlinger, 1991). These physiological changes are usually caused by such environmental cues as temperature, day length, moisture, and salinity (Leather et al., 1993; Ansart & Vernon, 2003).

Some molluscs increase their cold hardiness during cold weather (Ansart & Vernon, 2003). Several environmental factors enhance this ability. For example, cold acclimatization seems to be a major factor increasing the cold hardiness of an intertidal snail, *Melampus bidentatus* (Loomis & Hayes, 1987), and a terrestrial snail, *Anguispira alternata* (Riddle & Miller, 1988). Photoperiod and starvation are known environmental cues that increase the cold hardiness of the land snail *Helix* [*Cornu*] *aspersa* (Biannic & Daguzan, 1993; Ansart et al., 2001, 2002), and the cold hardiness mechanism of this species changes from freezing avoidance to freezing tolerance with increasing body size (Ansart & Vernon, 2004). A change in seawater salinity also increases the cold hardiness of some kinds of intertidal snails (Murphy, 1979). Although factors that enhance cold hardiness before winter are well studied, factors decreasing it are poorly understood.

The apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae) is a freshwater snail originating in tropical and temperate South America (Martin et al., 2001; Cowie, 2002). It was initially introduced into Asian countries as a source of human food. However, commercial markets failed, and escaped snails invaded rice paddies, becoming an important rice pest in many countries (Halwart, 1995; Wada, 2004; Cowie et al., 2006; Hayes et al., 2008). After the introduction this snail into temperate Japan in 1981, it became a pest of rice and an important constraint to the spread of direct sowing technology (Mochida, 1991; Wada et al., 1999; Wada,

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2004). Overwintered snails feed on sprouts or rice seedlings and seriously restrict seedling establishment, especially after heavy rain.

A recent study revealed that *P. canaliculata* increases its cold hardiness before the onset of winter (Wada & Matsukura, 2007), and cold acclimatization was a major factor enhancing it. Desiccation also enhanced it to an extent, and starvation might have increased it. However, photoperiod did not influence it (Matsukura & Wada, 2007). Glycerol content increased with the development of cold hardiness, and glucose content showed temporal increase (Matsukura et al., 2008). Both molecules are well known to increase cold hardiness in insects (Lee & Denlinger, 1991; Leather et al., 1993).

The purpose of this study was to determine the effects of temperature, water condition, and starvation on decreasing the cold hardiness of overwintering *P. canaliculata*. These ecological factors are known to influence the increase of cold hardiness in this species (Matsukura & Wada, 2007). It is necessary to understand how cold hardiness decreases as well as increases in order to fully understand the ecological and physiological mechanisms of cold hardiness change in *P. canaliculata*. This study is the first to experimentally determine the environmental factors that lower cold hardiness in molluscs. In addition, we examined the changes in glycerol and glucose contents during the loss of cold hardiness.

MATERIALS AND METHODS

Snails

We collected snails found resting on the sediment of channels around the lotus paddy fields in Kumamoto city, Kumamoto Pref., Japan (32.5°N, 130.4°E), on 1 December 2006. We used only juveniles with shell heights from 7.5 to 17.5 mm, which constitute the majority of overwintering snails in paddy fields (Wada et al., 2004). The cold hardiness of *P. canaliculata* is fully developed by December (Wada & Matsukura, 2007). Thus, the snails collected were cold-tolerant ones; we confirmed this with a mortality test.

Cold Treatment to Evaluate the Cold Hardiness of Snails (Mortality Test)

We evaluated the cold hardiness of the snails by the method as described in Wada & Matsukura (2007). Twenty snails (basically, five replicates) were wrapped in a moist towel, confined in a plastic cup (10 cm diameter, 4 cm deep), and held at 0°C for five days. Cold-intolerant snails (collected in summer) cannot survive this treatment, but most cold-tolerant snails (collected in December) can (Wada & Matsukura, 2007). Thus, cold-tolerant populations can be clearly distinguished from intolerant ones. After the treatment the snails were immersed in tap water for 24 h at room temperature (17–22°C). Snails that everted a foot were regarded as survivors.

Environmental Treatment 1: Temperature

Snails were reared at 25, 20, or 15°C. They were confined in a plastic container (18 cm × 30 cm, 20 cm deep; 100 snails per container) filled with ample water (approximately 15 cm deep) and given a cabbage leaf as food. The containers were kept under long-day condition (16 h light, 8 h dark). At 25°C, 100 snails were taken out of the container at 0.25 (6 h), 0.5 (12 h), 1, 2, and 4 days, respectively (totally 500 snails used), then cold-tested (mortality test). At 20°C, snails were taken out and tested at 0.25, 1, 4, and 8 days. At 15°C, snails were sampled as at 20°C and at 64 days. Thus, a total of 1,400 snails were used in this experiment.

Environmental Treatment 2: Water Condition

Because apple snails hibernate in water or in the soil of drained paddy fields, we compared the cold hardiness of snails between two water conditions: in aquatic and moist conditions. Twenty snails were wrapped in a moist towel, confined in a plastic cup, and held at 25°C as above. Survival was checked by mortality test at 1, 4, 8, and 64 days (4 replicates in each sampling date). We compared the survivorship with that at 25°C in treatment 1, which was conducted in aquatic condition.

Environmental Treatment 3: Starvation

A Starvation test in aquatic condition was conducted at 25°C. The 25°C condition in treatment 1 was used as the fed treatment. No food was given in the starved treatment. Survival was checked by mortality test at 0.25, 1, and 4 days (20 snails × 5 replicates in each sampling day).

Chemical Analyses

We measured the glucose and glycerol contents of snail bodies in the 25°C temperature of treatment 1. Snails were sampled at 0 (control), 0.25, 0.5, 1, 2, or 4 days. The soft body was carefully removed from the shell, weighted (fresh mass: FM), and homogenized in 2 mL 80% ethanol. The tissue was extracted according to Izumi et al. (2005). The glucose was measured by the mutarotase-GOD method (Glucose CII-test, Wako, Japan), as described in Matsukura et al. (2008). The glycerol was measured with an F-kit (Boehringer Mannheim, Mannheim, Germany). Ten snails were used in each measurement.

Statistical Analyses

Analysis of variance (ANOVA) followed by Tukey's Highly Significant Differences (HSD) test at 5% probability level were conducted for multiple comparisons of the survival in the mortality test following the temperature treatment. The *t*-test was used to compare the water conditions and the feeding conditions, and an ANOVA followed by Tukey's HSD test was used to compare glucose and glycerol contents between samples. Pearson's product-moment correlation coefficient (r) was estimated to evaluate the interdependence between the survival and the amounts of glucose and glycerol. All percentage data were arcsine square-root transformed before analysis.

Temperatures in Field

We obtained temperature data from Automated Meteorological Data Acquisition System (AMeDAS) point at Kikuchi, Kumamoto Pref. (32.9°N, 130.8°E) operated by the Japanese Meteorological Agency. We calculated the daily average, average of daily maximum, and average of daily minimum temperatures from December to June for 5 years (from 2002–2003 to 2006–2007).

RESULTS

Initial Cold Hardiness of Field-collected Snails

Before any treatments (just after collection), 83.0 ± 0.04% (mean ± SE) of snails survived the cold treatment (0°C for five days), indicating that most field-collected snails were cold tolerant.

Changes in Survival in the Mortality Test

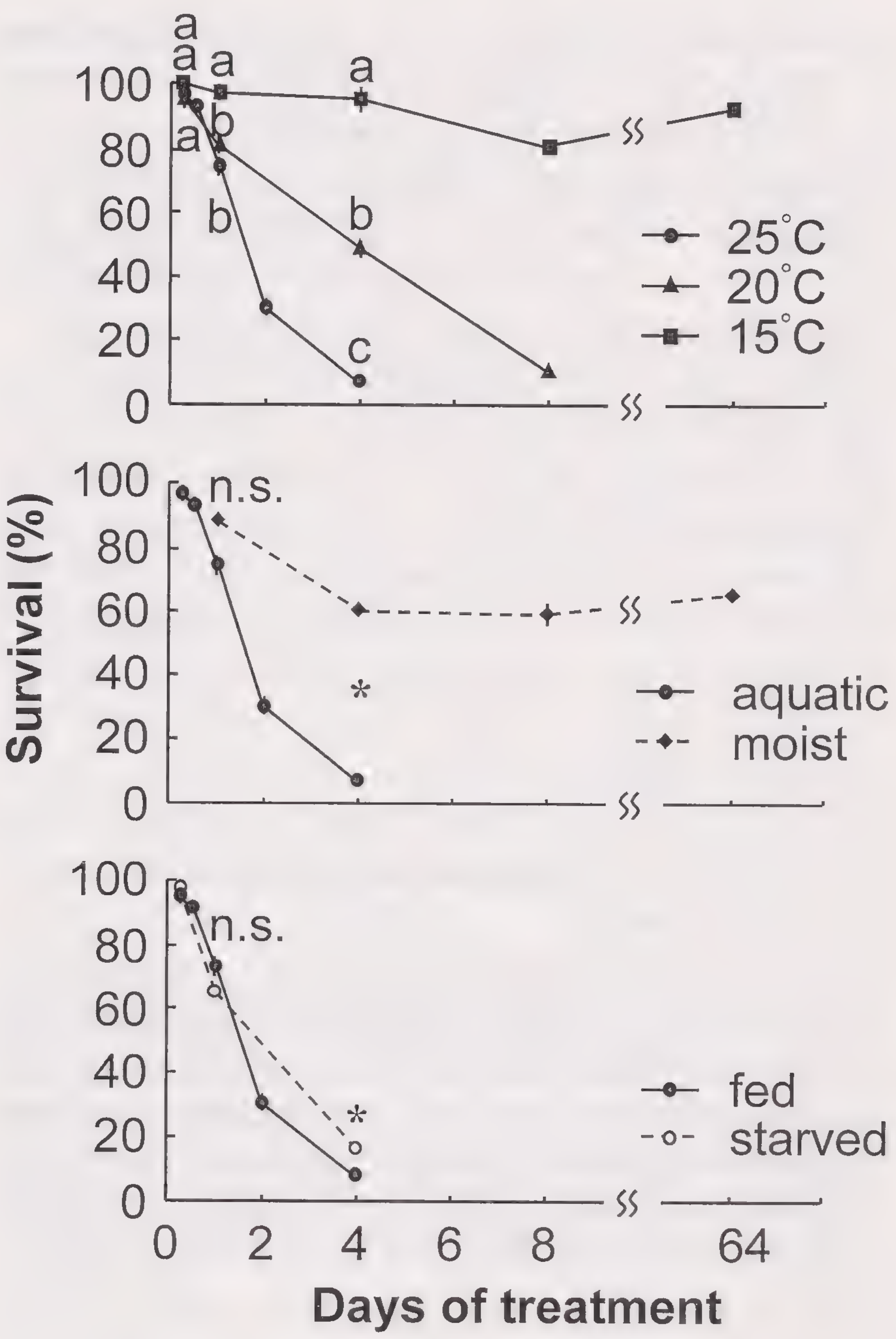

FIG. 1. Changes in survival after cold treatment (0°C for five days) of *P. canaliculata* reared under different environmental treatments: temperature in aquatic condition (top); water condition (center); starvation (bottom). Vertical bars indicate SE; a–c: Different letters indicate significant difference from other treatments on the same sampling date (Tukey's HSD test, $P < 0.05$). *Significant difference between two treatments on the same sampling date (*t*-test, $P < 0.05$).

Temperatures greatly affected survivals in environmental treatment 1 (Fig.1). More than 95% of snails held at each temperature for 0.25 day (6 h) survived cold treatment, and the difference in survivals depending on temperatures was not clear (ANOVA, $F = 1.229$, d.f. = 14, $P = 0.327$). At one day, however, survivals were affected by water temperatures ($F = 9.542$, d.f. = 14, $P = 0.003$), and survival at 25°C (74.0 ± 5.8%) and at 20°C (81.0 ± 4.3%) were significantly lower than that at 15°C (97.0 ± 1.2%) (Tukey's HSD test). At four days, the difference became more conspicuous ($F = 79.477$, d.f. = 14, $P < 0.001$). Survival was much lower at 25°C (7.0 ± 2.6%) and 20°C (49.0 ± 5.3%) than at 15°C (95.0 ± 2.2%), and the difference in survival was significant between any pair of temperatures (Tukey's HSD test). At eight days, survival at 20°C decreased to 10.0 ± 2.7%. These results indicate almost complete loss of cold tolerance after four days at 25°C

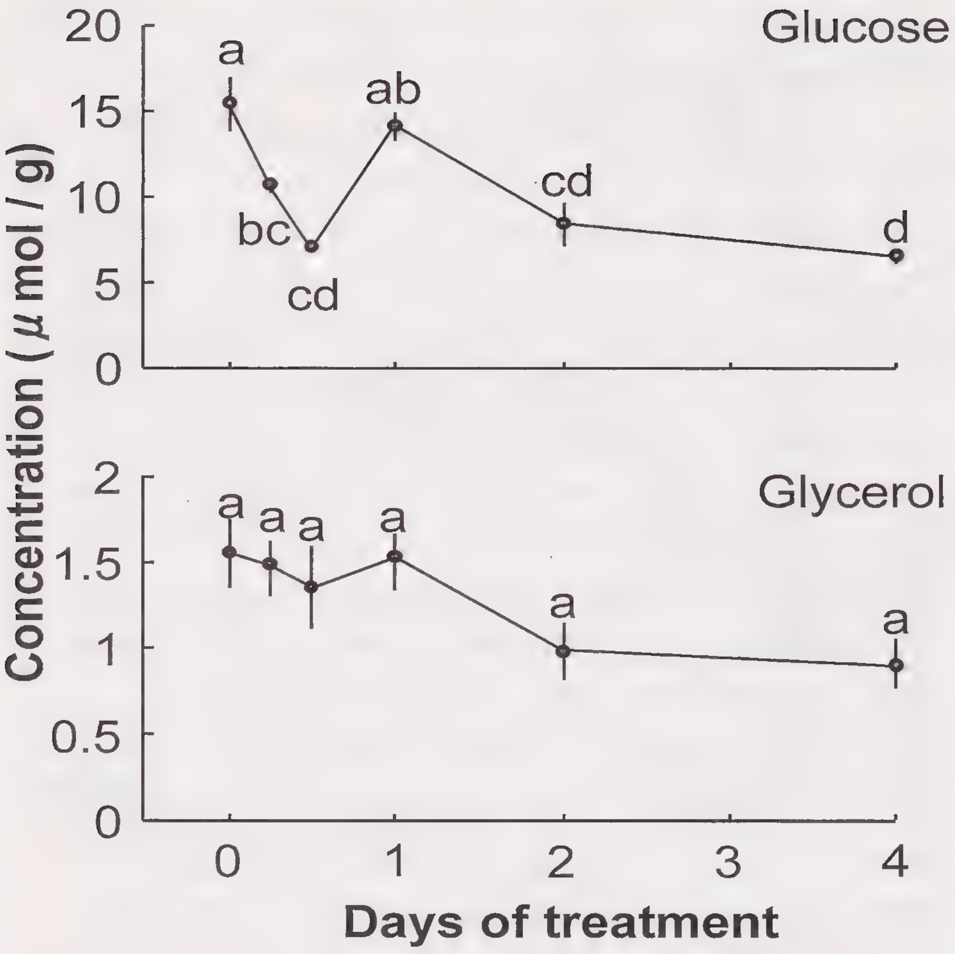

FIG. 2. Glucose (top) and glycerol (bottom) contents during the course of cold hardiness reduction in *P. canaliculata.* Snails were reared at 25°C in aquatic condition with food. Vertical bars indicate SE. Different letters indicate significant difference between samples (Tukey's HSD test, $P < 0.05$). Ten snails were used on each sampling date.

and after eight days at 20°C. On the other hand, snails reared at 15°C retained high survivorship (92.0 ± 1.2%) until 64 days.

At one day, there was no significant difference in survival of snails after cold treatment between the aquatic (74.0 ± 5.8%) and moist (87.5 ± 3.2%) conditions at 25°C (*t*-test, $t=2.36$, d.f. = 8, $P = 0.137$) (Fig. 1). However, at four days, survival in moist storage (60.0 ± 11.4%) was significantly higher than that in aquatic condition (7.1 ± 2.6%) ($t = 2.365$, d.f. = 8, $P = 0.002$). The survival of snails in moist storage remained around 60% up to 64 days, indicating the retention of cold hardiness.

At four days, the survival of starved snails decreased to 16.0 ± 1.9%, and that of fed snails decreased to 7.0 ± 2.6% (Fig. 1). The survival of starved snails was slightly but significantly higher ($t=2.31$, d.f. = 9, $P = 0.041$).

Changes in Glucose and Glycerol Contents During Treatment

Glucose contents varied greatly (ANOVA, $F = 15.327$, d.f. = 5, $P < 0.001$), between a maximum of 3.1 ± 0.3 μg/mg FM at 0 days and a minimum of 1.3 ± 0.1 μg/mg FM at four days at 25°C in aquatic condition (Fig. 2). However, the trend lacked consistency, decreasing gradually from 0 to 0.5 days, increasing suddenly at one day, and again decreasing gradually to four days (Fig. 2). There was no correlation between glucose content and survival (Pearson's regression analysis, r = 0.441, d.f. = 4, $P = 0.382$).

There was significant change in glycerol content (ANOVA, $F = 2.426$, d.f. = 5, $P = 0.047$), decreasing from a maximum of 0.14 ± 0.02 μg/mg FM at 0 days to a minimum of 0.08 ± 0.01 μg/mg FM at four days (Fig. 2). Furthermore, there was a significant positive correlation between the glycerol content and survival after the mortality test (Pearson's regression analysis, r = 0.879, d.f. = 4, $P = 0.021$).

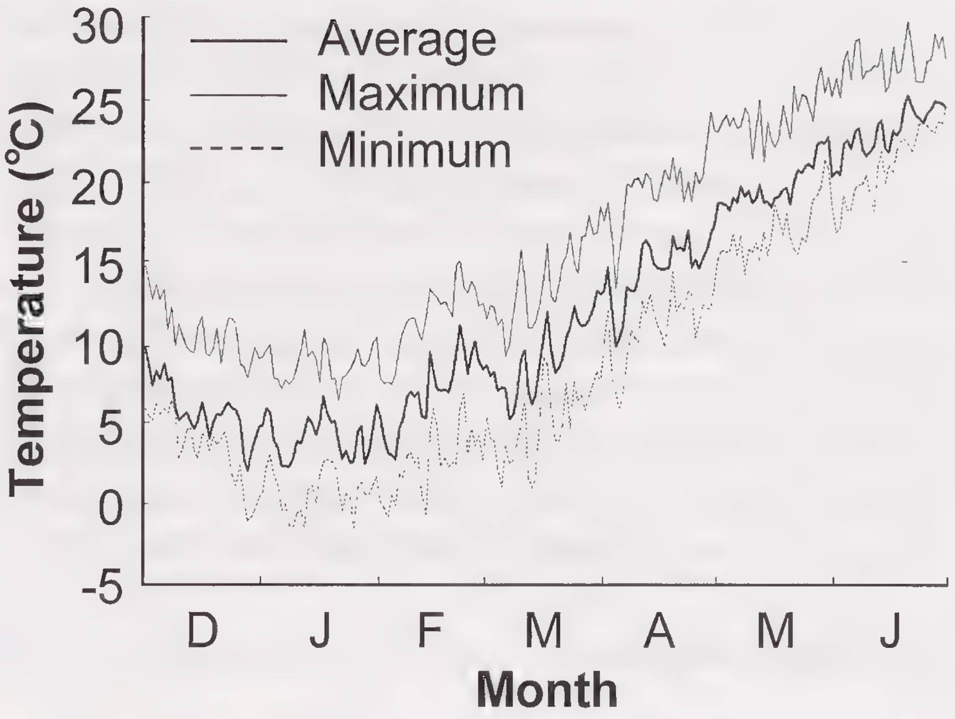

FIG. 3. Daily average, average of daily maximum, and average of daily minimum air temperatures from January to June at Kikuchi, Kumamoto, calculated from the past five years' data (2003–2007).

Field Temperature from January to June

The daily average temperature in Kikuchi was under 10°C on most days from December to mid-March, then it increased to 15°C in mid-April (Fig. 3). It reached nearly 20°C in early May and 25°C in mid-June.

DISCUSSION

Warm temperature greatly decreases cold hardiness in *P. canaliculata* in aquatic condition (Fig. 1). Cold hardiness was almost completely lost by four days at 25°C and by eight days at 20°C. Yet it was retained for 64 days at 15°C. These results indicate that the loss of cold hardiness in *P. canaliculata* depends on temperature ≥ 20°C in aquatic condition. Although environmental factors decreasing cold hardiness in molluscs have not previously been studied, exposition to warm temperature (deacclimatization) is known to lower cold hardiness in some insects (e.g., Rojas et al., 1983; Coulson & Bale, 1990; Šlachta et al., 2002) and plants (reviewed by Kalberber et al., 2006).

Water condition also affected the loss of cold hardiness. Although the snails lost their cold hardiness by four days at 25°C in aquatic condition, approximately 60% survived cold treatment after moist storage for at least 64 days (Fig. 1). These results show that the cold hardiness of *P. canaliculata* is not lost at warm temperature in moist storage. This fact supports the finding that moist storage encouraged cold-intolerant snails to acquire cold hardiness even at warm temperature (Matsukura & Wada, 2007). Cold hardiness and desiccation tolerance of insects often overlap physiologically and ecologically (Ring & Danks, 1994; Danks, 2000). Therefore, the response of *P. canaliculata* to desiccation – in fact, water content of snails decreased during moist condition (Matsukura et al., 2008) – suggests the linkage between cold hardiness and desiccation tolerance.

The starved snails survived the mortality test better than the fed snails at four days, however, difference in survival was slight (Fig. 1). Thus, starvation seems to be a minor factor to affect cold hardiness.

Our results support the previous suggestion that glycerol plays a major role in the cold hardiness of *P. canaliculata* (Matsukura et al., 2008). The glycerol content was positively correlated with survival in the mortality test, although its change was small (Fig. 2). The slight change may be due to the accumulation of glycerol in particular organs of cold-tolerant *P. canaliculata* (Matsukura et al., 2008). On the other hand, glucose content was not correlated with survival, and varied greatly during storage (Fig. 2). This result suggests that glucose does not directly affect the cold hardiness of *P. canaliculata*, but its content fluctuates as a result of the metabolic processes of cold hardiness change, as Matsukura et al. (2008) has suggested.

Our results indicate that the cold hardiness of *P. canaliculata* decreases within a week at 20°C in aquatic condition. This would mean that the cold hardiness of overwintering *P. canaliculata* living in water breaks down from late April to early May, because daily-average temperature reaches approximately 20°C until this period (Fig. 3). However, snails living in drained paddy fields would retain their cold hardiness until early summer. In Kumamoto, paddy fields are drained in autumn, so snails overwinter in moist or dry condition during winter to spring. In mid- to late June, the fields are irrigated for rice planting, and the cold hardiness of the snails would break down quickly then.

This study and our earlier studies (Wada & Matsukura, 2007; Matsukura & Wada, 2007) clearly revealed seasonality of cold hardiness in *P. canaliculata*. This seasonality may occur because of the relation between cost for acquiring cold hardiness and its benefits. Actually in some insects, synthesis of cold-hardiness-related molecules is reported to be costly, affecting their fecundity (Leather et al., 1993). In *P. canaliculata*, acquiring cold hardiness is probably accompanied by consumption of glycogen reserves (Matsukura et al., 2008). Therefore, *P. canaliculata* may break down its cold hardiness in summer, maximizing multiplication. Physiologically, there may be a trade-off between cold hardiness and heat tolerance. The land slug *Lehmannia valentiana* develops cold hardiness in winter, but its heat tolerance then inversely decreases (Udaka et al., 2008). Therefore, physiological state of cold- tolerant slugs has disadvantage for survivals in summer. Seasonality of cold hardiness in *P. canaliculata* may be attributed to this physiological trade-off.

Recently, new molecular approaches are applied in the study of physiological mechanisms of cold hardiness in insects. For example, replacement of water by glycerol in fat body cells with aquaporin, which is one kind of membrane transporter, prevents freezing injury of cells in *Chilo suppressalis* (Izumi et al., 2006). Unsaturated fatty acids produced by delta 9-acyl-CoA desaturases on fatty acid cells enhance cold hardiness in *Delia antique* (Kayukawa et al., 2007). On the contrary, such approaches have not been adopted to molluscs probably because of difficulty in mass rearing and poorly accumulated information in molluscs. *Pomacea canaliculata* seems to be a suitable animal for investigating the mechanisms, because basic knowledge about the cold hardiness of this species has been gradually made clear (e.g. Wada & Matsukura, 2007; Matsukura et al., 2008; this study). Large numbers of snails can be easily collected from paddy fields, and mass rearing is relatively easier than other molluscs. Further studies on the physiology of the cold hardiness in *P. canaliculata* will help to clarify the inclusive mechanism of cold hardiness in molluscs.

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THE GENERA *THYASIRA* AND *PARATHYASIRA* IN THE MAGELLAN REGION AND ADJACENT ANTARCTIC WATERS (BIVALVIA: THYASIRIDAE)

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ABSTRACT

This study provides a revision of the Magellanic and Antarctic species of *Thyasira* and *Parathyasira*. The status of all species previously reported from the area is revised and a new species described. Five species are recognized as valid: *Thyasira falklandica* (Smith, 1885); ***Thyasira scotiana***, n. sp.; *Thyasira debilis* (Thiele, 1912), new combination; *Parathyasira magellanica* (Dall, 1901), new combination; and *Parathyasira dearborni* (Nicol, 1965), new combination. *Cryptodon fuegiensis* Dall, 1890, previously reported under the genus *Thyasira*, is reallocated into *Conchocele* Gabb, 1866. *Loripes pertenuis* Smith, 1881, and *Thyasira bongraini* Lamy, 1911, are considered *nomen dubia*.

Key words: Magellan Region, Southern Ocean, Thyasiroidea, diversity.

INTRODUCTION

Thyasiridae Dall, 1901, is a world wide family of infaunal bivalves, occurring from shallow to hadal waters, including oxygen-poor and hydrogen sulfide-rich environments. In recent years, several studies contributed to the knowledge of this family: numerous new taxa were described and a number of taxonomic problems concerning the identity of several species clarified (e.g., Payne & Allen, 1991; Oliver & Killeen, 2002; Oliver & Sellanes, 2005; Oliver & Holmes, 2006; Barry & McCormack, 2007). However, as stated by Payne & Allen (1991) and Oliver & Killeen (2002), knowledge of the total diversity of the family is far from complete. This limitation arises not only from the difficulty in defining some of the taxa, but also from the few studies of species in some geographic areas. This is the case, for instance, for the Magellanic and Antarctic waters, from which seven species have been reported. The first described for this area, *Cryptodon falklandicus*, was named by Smith (1885), from the Malvinas (Falkland) Islands; afterwards, Dall (1890) described *Cryptodon fuegiensis* from the Magellan Strait, and subsequently *Thyasira magellanica* Dall, 1901, from the west coast of Patagonia. Later, Lamy (1911a) described *Axinus bongraini* based on a specimen from the Antarctic Peninsula, and Thiele (1912) described *Axinopsis debilis* from the Davis Sea. More recently, Nicol (1965) described the sixth species for the area, *Thyasira dearborni*, from the Ross Sea. Furthermore, *Loripes pertenuis* Smith, 1881, was regarded as a thyasirid by Soot Ryen (1959).

The description of all the Magellanic and Antarctic species was based on shell characters of few specimens, if not on a single valve, as in the case of *Thyasira magellanica*, *Axinus bongraini*, and *Axinopsis debilis*. These descriptions disregarded such characters as the posterior area of shell (i.e., the presence / absence of auricle, development of folds and sulcus) or tubercles on hinge plate, which are important for species identification. None of the species was subsequently redescribed, and intraspecific variability was never examined. In addition, the anatomy of these species has been completely unknown. Under these conditions it is not strange that species have been frequently confused, and numerous synonymies erroneously inferred.

This paper undertakes a systematic revision of the thyasirid species occurring in the Magellan Region and adjacent Antarctic waters. All thyasirid species are redescribed and illustrated, their intraspecific variability examined, and gross anatomy studied.

MATERIAL AND METHODS

This study is based on the material collected during several field trips at the Beagle Channel, Magellan Strait, Isla de los Estados, and the Scotia Arc Islands. Specimens were collected with a dredge of 2 mm mesh size net, at depths between 5 and 587 m, and sorted from the sediment using stereoscopic microscopy. Additional specimens came from the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Argentina (MACN-In); Museo de La Plata, Argentina (MLP); National Museum of Scotland, Great Britain (NMSZ); National Institute of Water and Atmospheric Research, New Zealand (NIWA); Zoologisches Institut und Zoologisches Museum der Universität Hamburg, Germany (ZMH); and Bavarian State collection of Zoology, Germany (ZSM). All traceable type specimens housed at The Natural History Museum, Great Britain (NHM); the United States National Museum, Smithsonian Institution, USA (USNM); the Muséum National d'Histoire Naturelle, France (MNHN); and the Museum für Naturkunde, Germany (ZMB), were studied.

Shell morphology was studied and illustrated using scanning electron microscopy (SEM). Linear measurements were taken according to the following criteria: L = maximum anteroposterior distance; H: maximum dorsoventral distance, perpendicular to L; W: maximum distance across the united valves. The mean values and standard deviations for the W/H ratio, and the number of measurements (n) are given. The number of specimens (sp/sps) and valves (v/vs) from each sampling station was also indicated.

For anatomical descriptions, specimens were decalcified by rinsing in 5% formaline with 2% acetic acid. Specimens were dissected under a stereoscopic microscope. The nomenclature of the soft parts anatomy and shell morphology follows Oliver & Killeen (2002); the description of the digestive gland refers to the shape of the lateral body expansions ("pouches").

Due to the numerous species misidentifications, the synonymies included in this study do not encompass the total literature for each species but only those cases for which it was possible to confirm the identity of the referred specimens.

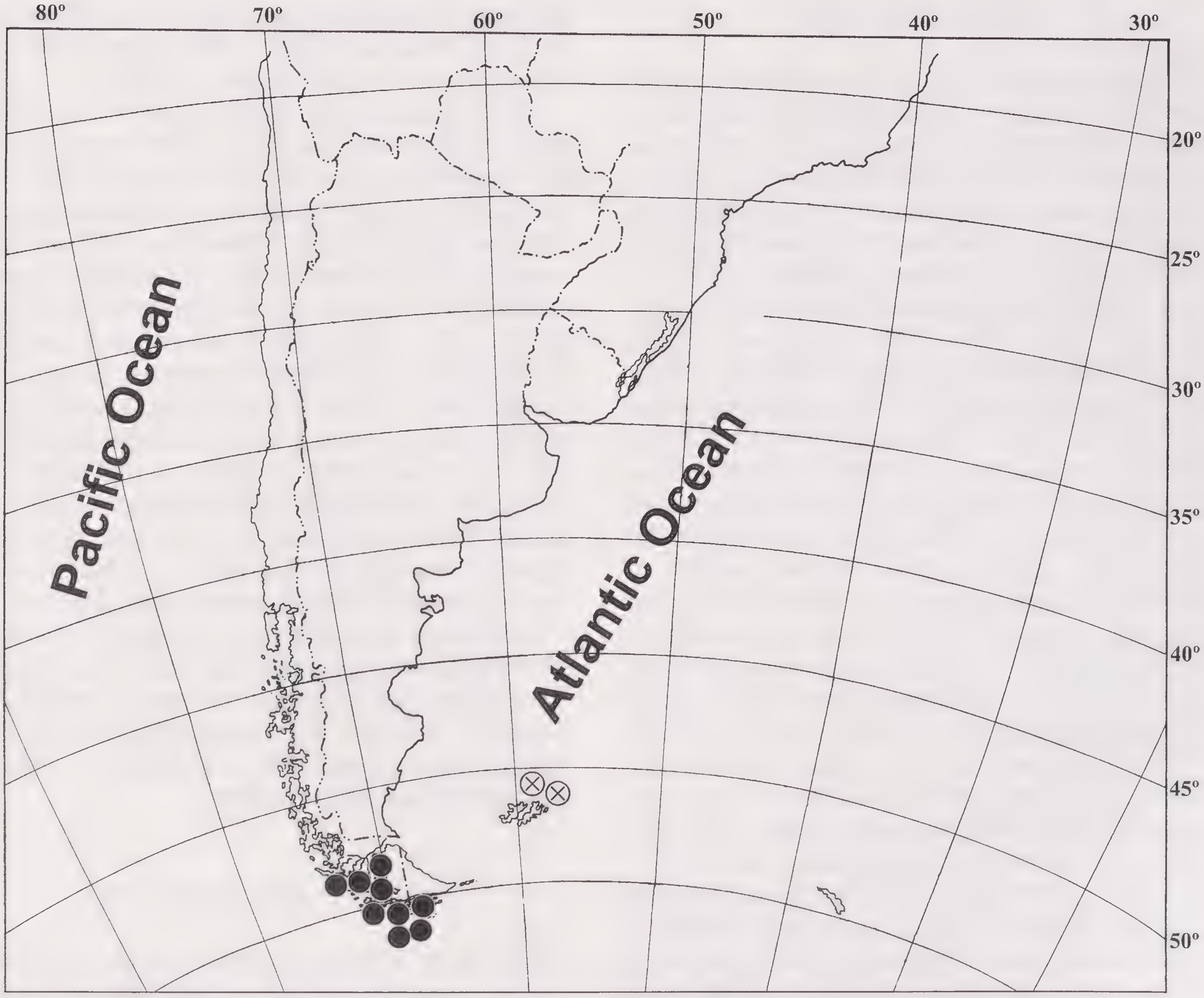


FIG. 1. Known distribution of *Thyasira falklandica* (⊗) and *Thyasira magellanica* (●).

SYSTEMATICS

Thyasiridae Dall, 1901

Thyasira Lamarck, 1818 (ex Leach, MS)
Type Species: *Tellina flexuosa* Montagu, 1803 (by monotypy)

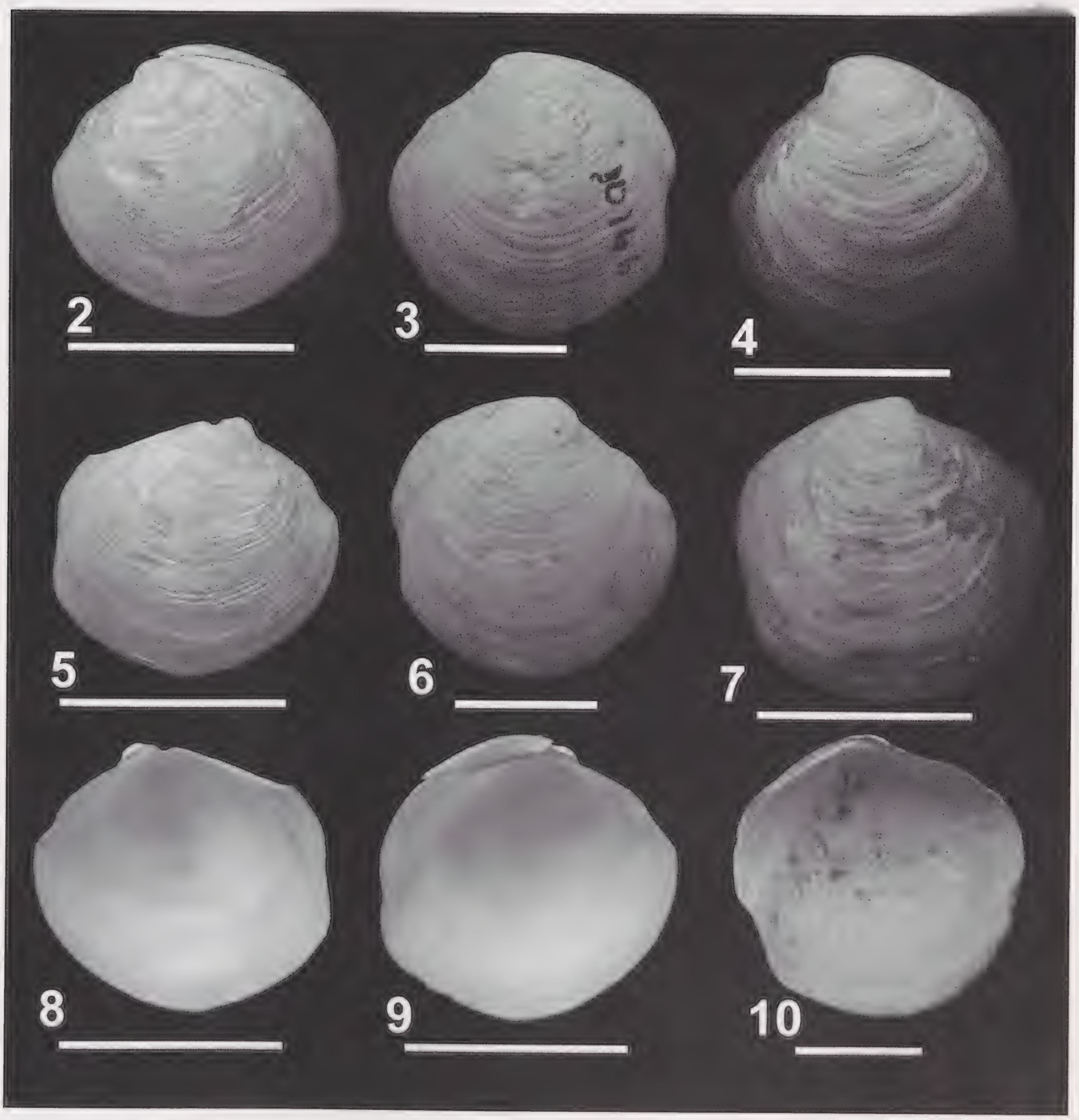
Thyasira falklandica (Smith, 1885)

Figures 1–10

Cryptodon falklandicus Smith E. A., 1885: 190, pl. 14, fig. 3, 3a.
Thyasira falklandica: Lamy, 1921: 300.

Type Locality

Station 316 R.V. Challenger [51°32'S, 58°06'W], off Falkland [Malvinas] Islands, 3–5 fms [5.5–9.2 m].



FIGS. 2–10. *Thyasira falklandica*. FIGS. 2, 5, 8, 9: One of the two syntypes (NHM 1887.2.9.2791-2); FIGS. 3, 4, 6, 7, 10: Specimens from Islas Malvinas (MACN-In 10166); FIGS. 2–4: Outer view left valve; FIGS. 5–7: Outer view right valve; FIG. 8: Inner view right valve; FIGS. 9, 10: Inner view left valve. Scale bars = 10 mm.

Material Examined

Two syntypes (NHM 1887.2.9.2791-2); Islas Malvinas [51°27'S, 59°00'W] (2 sps, MACN-In 10166; 2 sps, NHM 1963360).

Distribution

Only known with certainty from Malvinas (Falkland) Islands (Fig. 1).

Description

Shell large (maximum observed H = 19 mm), solid, inflated (W/H = 0.62 ± 0.06, n = 3), subovate, nearly equilateral. Anterior end markedly pointed (Figs. 2–7). Anterior part of dorsal margin moderately long, sloping downwards straight or slightly concave near beaks, flattened distally. Anterior and ventral margins evenly rounded; sharp angle marking joining point of dorsal and anterior margins (Figs. 2–7). Ventral margin strikingly curved, sometimes slightly projecting in middle. Lunule wide. Posterior area of shell sulcate, producing variably marked posterior sinus; first and second posterior folds low, rounded (Figs. 2–7). Submarginal sulcus and marginal sinus not defined. Ligament internal, externally visible. Beaks low, wide, subcentral, anteriorly directed. Shell surface whitish, dull, sculpture represented by prominent growth lines. Periostracum thin, yellowish buff. Hinge plate narrow, edentulous (Figs. 8–10).

Remarks

The anatomy of *Thyasira falklandica* is unknown. However, based on the general shell morphology, the species is tentatively retained under *Thyasira*. Dias Passos et al. (2007) provided information on the anatomy of a thyasirid from South Shetland Islands, which they referred to as *Thyasira falklandica*; but from the illustrations they provided, the identification does not seem correct.

Melvill & Standen (1907) reported specimens from South Orkney as *Thyasira falklandica*. Re-examination of this material (NMSZ 1921.143.715) showed shells less inflated than those of topotypic specimens of *T. falklandica*, with the anterior end more projecting, an evenly rounded anterior margin, and a shallower posterior sulcus. Furthermore, in the specimens from South Orkney, the lunule is less evident and the shell surface shiny. This material seems to be conspecific with that figured by Oliver & Sellanes (2005, figs. 35–38) as *Thyasira falklandica*.

Thyasira falklandica is most similar to ***Thyasira scotiana***, from which it differs by having a larger, heavier, and more inflated shell, with ovate shell outline, and deeper posterior sulcus.

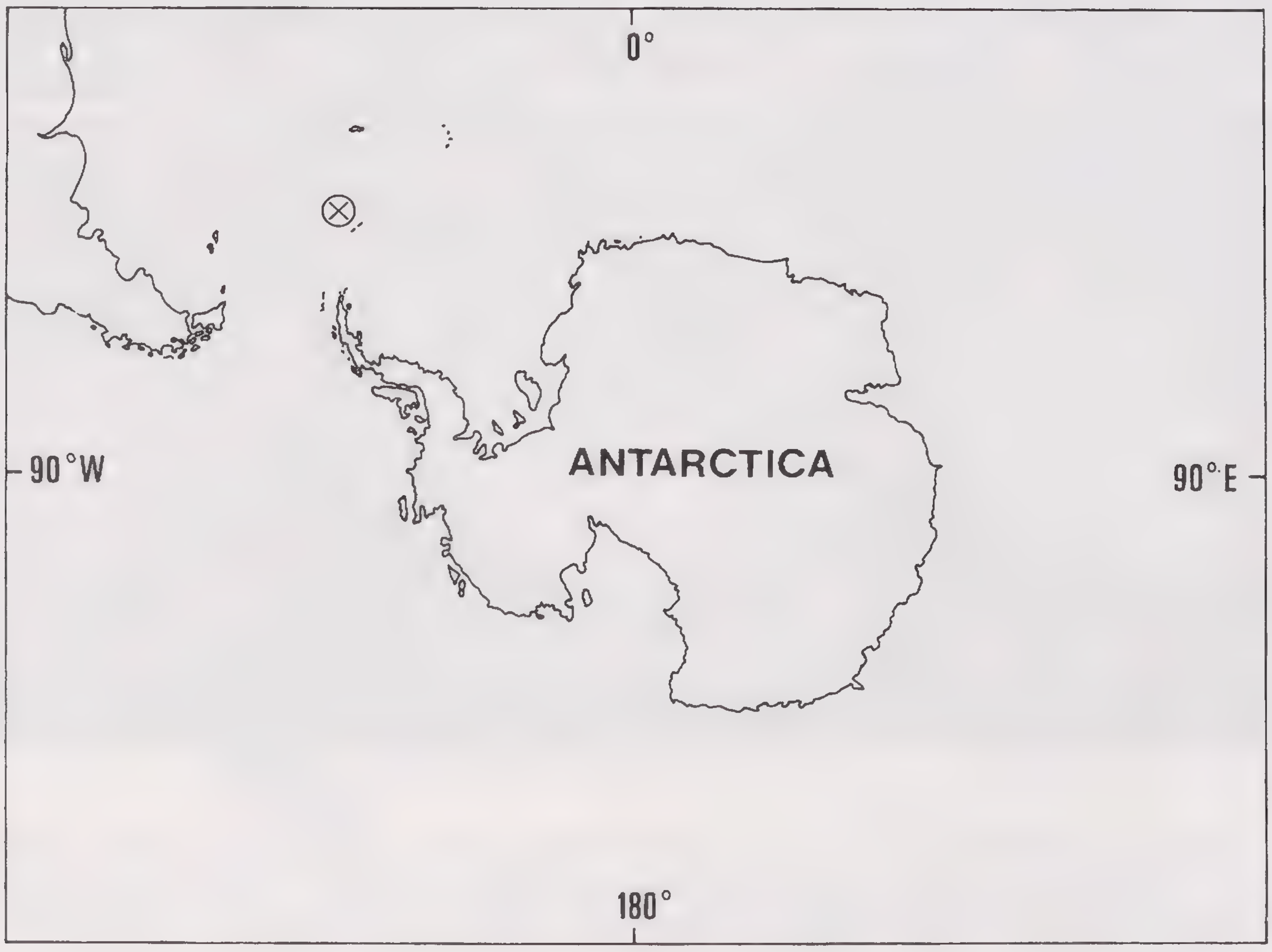


FIG. 11. Known distribution of ***Thyasira scotiana***.

***Thyasira scotiana*, n. sp.**
Figures 11–20

Type Locality

South Orkney Islands [60°35'S, 45°30'W] (Fig. 11).

Type Material

Holotype (MLP 5566-1) and 17 paratypes (MLP 5566-4).

Etymology

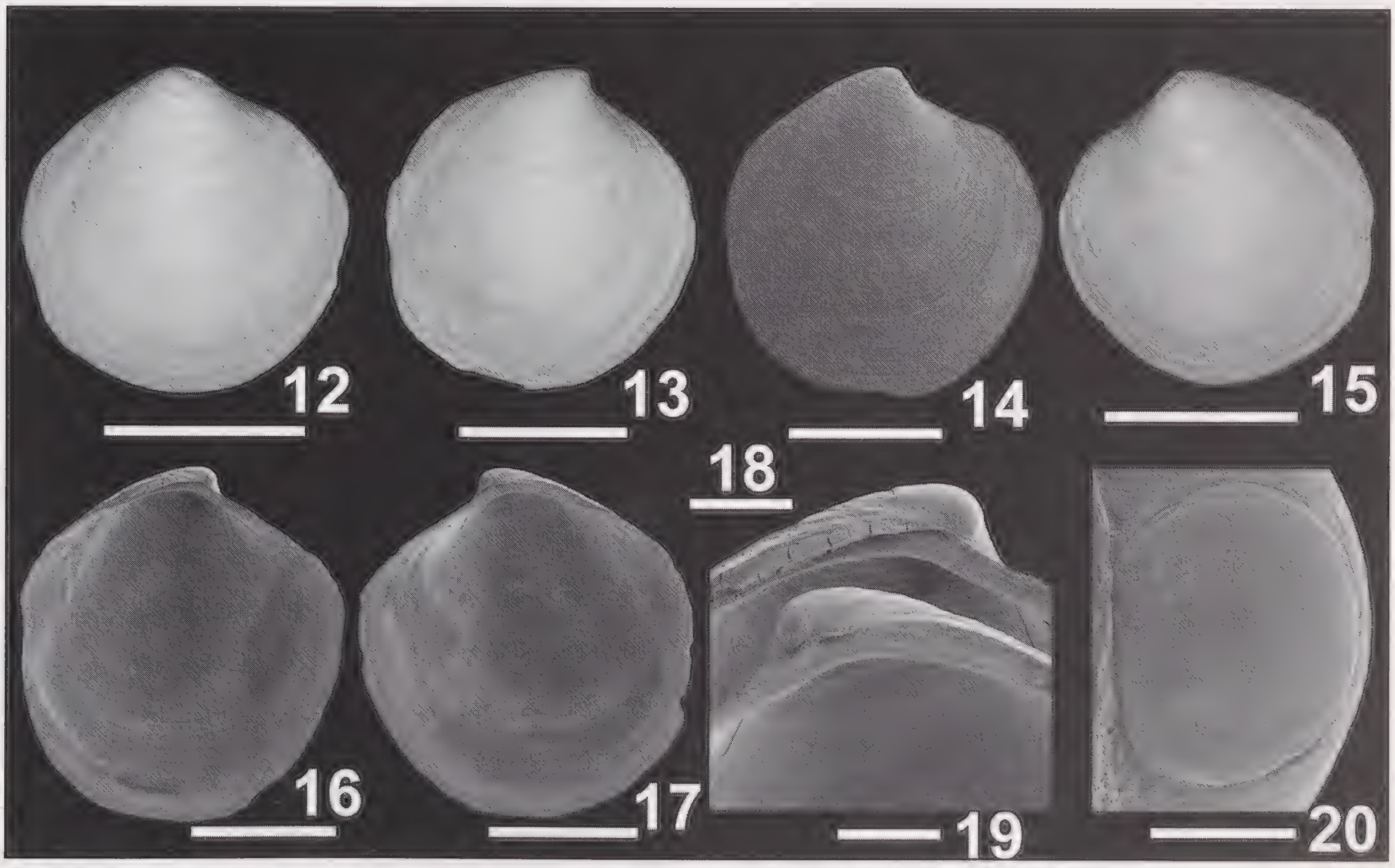
The species is named after the Scotia Sea, the geographic area of collection.

Diagnosis

Thyasira scotiana is characterized by a flat shell, with subcircular shell outline, strong first posterior fold, and large protoconch, of about 210 μm in diameter.

Description

Shell large (maximum observed H = 10 mm), approximately equilateral, subcircular, flat (W/H = 0.49 ± 0.02, n = 10), solid (Figs. 12–15). Anterior end rounded to slightly projecting. Anterior part of dorsal margin long, sloping downwards straight, forming a marked angle when joining anterior margin (Figs. 12–17). Anterior margin forming wide oblique curve with anterior portion of ventral margin, giving appearance of being distorted; posterior part of ventral margin rounded (Figs. 12–17). Sometimes ventral margin subangulate at junction of anterior and posterior parts (Fig. 15). Posterior area of shell weakly sulcated. Posterior margin truncated. First posterior fold wide, strong; second posterior ill defined (Figs. 12–15). Submarginal sulcus and marginal sinus not defined. Beaks low, acute, subcentrally located, anteriorly directed. Protoconch about 210 μm diameter; initial part sculptured with several folds, symmetrically distributed with respect to main central axis (Fig. 20). Shell surface whitish,



FIGS. 12–20. ***Thyasira scotiana***. FIG. 12: Holotype (MLP 5566-1); FIGS. 13–20: Paratypes (MLP 5566-4); FIGS. 12–14: Outer view right valve; FIG. 15: Outer view left valve; FIG. 16: Inner view left valve; FIG. 17: Inner view right valve; FIGS. 18, 19. Detail of hinge. FIG. 18: Left valve; FIG. 19: Right valve; FIG. 20: Protoconch. Scale bars FIGS. 12, 13, 15 = 5 mm; FIGS. 14, 16, 17 = 2 mm; FIGS. 18, 19 = 500 μm; FIG. 20 = 100 μm.

dull, sculptured with markedly and irregularly distributed growth lines. Periostracum pinkish cream. Hinge plate narrow, slightly enlarged below beaks, edentulous (Figs. 18, 19). Ligament internal but externally visible. Lunule not well defined.

Anatomy: Inner and outer demibranchs, present. Transverse section of anterior adductor muscle larger than posterior muscle (other anatomical characters were not possible to study as specimens were dried).

Remarks

Thyasira scotiana closely resembles *Thyasira falklandica*, differing in having a more rounded shell outline, a flatter and thiner shell, and a shallower posterior sulcus. The shell outline and larger protoconch also distinguish **Thyasira scotiana** from other species known from the area.

Thyasira debilis (Thiele, 1912), new combination

Figures 21–34

Axinopsis debilis Thiele, 1912: 232, pl. 18, fig. 25, 25a.

Genaxinus debilis: Dell, 1964: 208; Egorova, 1982: 70, figs. 314, 315; Dell, 1990: 57, figs. 93, 94.

Genaxinus bongraini: Nicol, 1966: 63 [in part], pl. 6, figs. 1, 2 [*non* Lamy, 1911].

Thyasira bongraini: Mühlenhardt Siegel, 1989: 162 [in part], pl. 13, fig. 24 [*non* Lamy, 1911].

Type Locality

Gauss Station [66°S, 89°E, Davis Sea, Antarctica].

Material Examined (Fig. 21)

Holotype (ZMB 63112). Ross Sea: 77°27’S, 172°22’E, 752 m (3 sps & 4vs, NIWA 45844); 77°05’S, 177°12’E, 362 m (7 sps & 31 vs, NIWA 45845); 76°42’S, 179°44’E, 318–472 m (1 sp, NIWA 45846); 74°30’S, 179°40’E, 238–301 m (13 sps & 33 vs, NIWA 45847); 77°30’S, 164°38’E, 256–260 m (2 sps, NIWA 45848); 77°50’S, 166° 30’E, 377 m (2 sps & 6 vs, NIWA 45849). Bellingshausen Sea: 70°52’52”S, 98°26’07”W, 425 m (5 sps, MLP 12703). Weddell Sea: 71°07’30”S, 11°27’42”W (6 sps, ZSM 20013026); 71°12’24”S, 12°26’48”W (2 sps, ZSM 20012843); 71°08’54”S, 13°12’48”W (4 sps, ZSM 20012835); 71°06’12”S, 12°50’27”W (1 sp, ZSM 20012831; 1 sp, ZSM 20012841). Antarctic Peninsula: 63°30’S, 54°15’W, 200 m (79 sps, ZMH 274; 120 sps, ZMH 275); 63°11’30’’S, 58°47’00’’W, 93 m (155 sps, ZMH 287; 76 sps, ZMH 302); 66°36’06’’S, 68°41’54”W, 607 m (4 sps, ZMB 114673); 65°54’54”S, 66°52’18”W, 175 m (83 sps, ZMH 357; 70 sps, ZMH 358); 66°32’06’S, 68°30’06’’W, 500 m (1 sp, ZMH 360); 65°04’30”S, 66°58’54”W, 269 m (2 sps, ZMH 994); 66°09’30”S, 67°19’00”W, 375 m (6 sps, ZMH 995); 66°39’18”S, 69°23’36”W, 344 m (10 sps, ZMH 996); 66°31’12”S, 68°25’18”W, 464 m (2 sps, ZMH 32961). South Shetland Islands: 61°23’27”S, 55°26’49”W, 285–288 m (581 sps, MLP 12704); 61°15’S, 55°00’W, 120 m (23 sps, ZMH 276); 62°51’S, 56°00’W, 133 m (28 sps, ZMH 277; 2 sps, ZMH 279; 98 sps, ZMH 280; 137 sps, ZMH 301); 60°53’S, 56°45’W, 485 m (45 sps, ZMH 281); 62°09’36’’S, 58°21’00’’W, 485 m (8 sps, ZMH 286); 62°05’18”S, 57°39’00”W, 265 m (90 sps, ZMH 282); 62°59’S, 57°05’W, 68 m (97 sps, ZMH 285); 61°13’48”S, 54°39’12”W, 280 m (377 sps, ZMH 306); 61°18’30”S, 54°40’18”W, 370 m (84 sps, ZMH 308; 45 sps, ZMH 309); 61°21’48”S, 56°00’36”W, 368 m (140 sps, ZMH 310; 202: sps, ZMH 311); 61°09’42”S, 56°10’18”W, 290 m (63 sps, ZMH 314); 61°12’42”S, 55°56’24”W, 134 m (15 sps, ZMH 317; 10 sps, ZMH 318; 274 sps, ZMH 319; 111 sps, ZMH 320); 61°09’00”S, 56°07’12”W, 208 m (61 sps, ZMH 321; 222 sps, ZMH 322); 61°03’54’’S, 55°58’48”W, 262 m (190 sps, ZMH 323; 160 sps, ZMH 324); 60°57’06”S, 55°55’12”W, 246 m (4 sps, ZMH 327); 60°51’18”S, 55°45’36”W, 290 m (24 sps, ZMH 334); 60°50’54”S, 55°37’24”W, 242 m (13 sps, ZMH 336; 428 sps, ZMH 338); 61°00’18”S, 55°13’36”W, 195 m (24 sps, ZMH 342; 50 sps, ZMH 343; 45 sps, ZMH 344); 60°53’36”S, 55°46’06”W, 260 m (6 sps, ZMH 345; 61 sps, ZMH 346); 62°49’18”S, 60°13’30”W, 850 m (3 sps, ZMH 349); 63°43’00”S, 61°13’36”W, 180 m (56 sps, ZMH 351); 62°46’36”S, 60°54’48”W, 140 m (56 sps, ZMH 352); 63°16’48”S, 63°44’30”W, 340 m (21 sps, ZMH 354; 36 sps, ZMH 355); 61°00’42”S, 55°01’24”W, 247 m (102 sps, ZMH 980); 60°56’24”S, 55°40’00’W, 112 m (33 sps, ZMH 981; 11 sps, ZMH 982; 95 sps, ZMH 983; 99 sps, ZMH 984); 61°13’18”S, 55°42’48”W, 96 m (4 sps, ZMH 986); 62°49’30”S, 60°57’30”W, 184 m (67 sps, ZMH 988; 28 sps, ZMH 989; 4 sps ZMH 990; 28 sps, ZMH 991); 63°18’00”S, 63°42’48”W, 300 m (21 sps, ZMH 993);

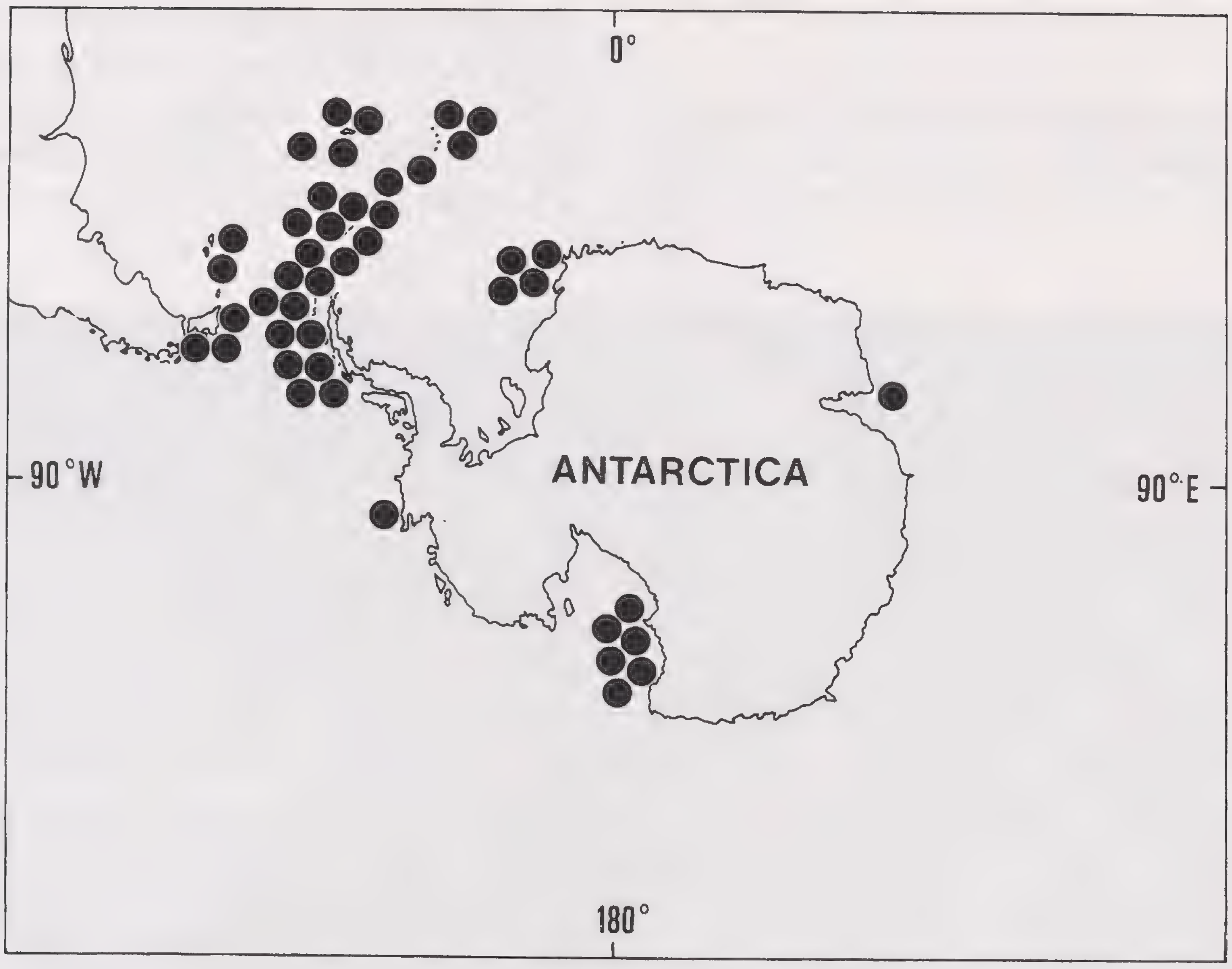
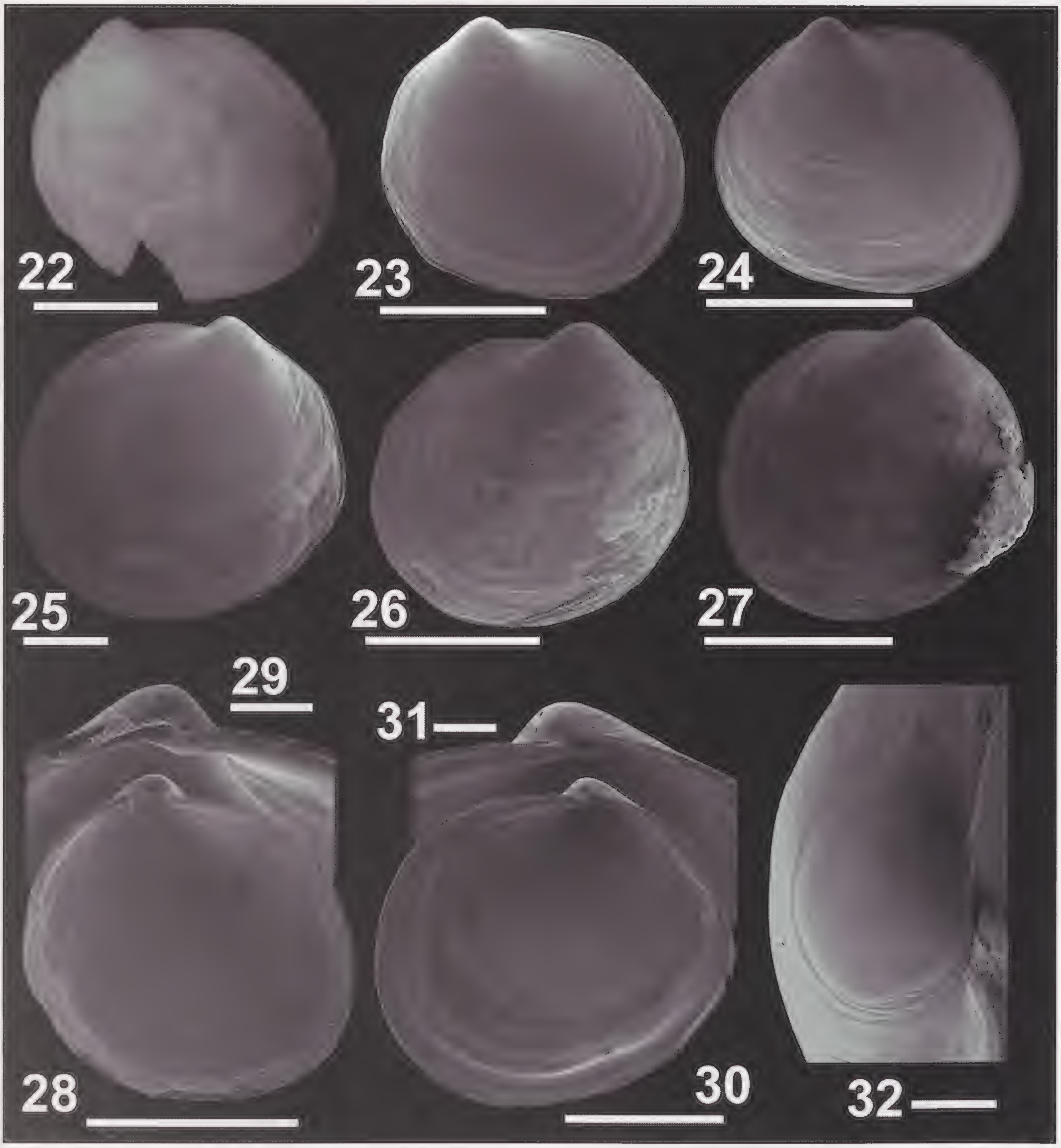


FIG. 21. Known distribution of *Thyasira debilis*.

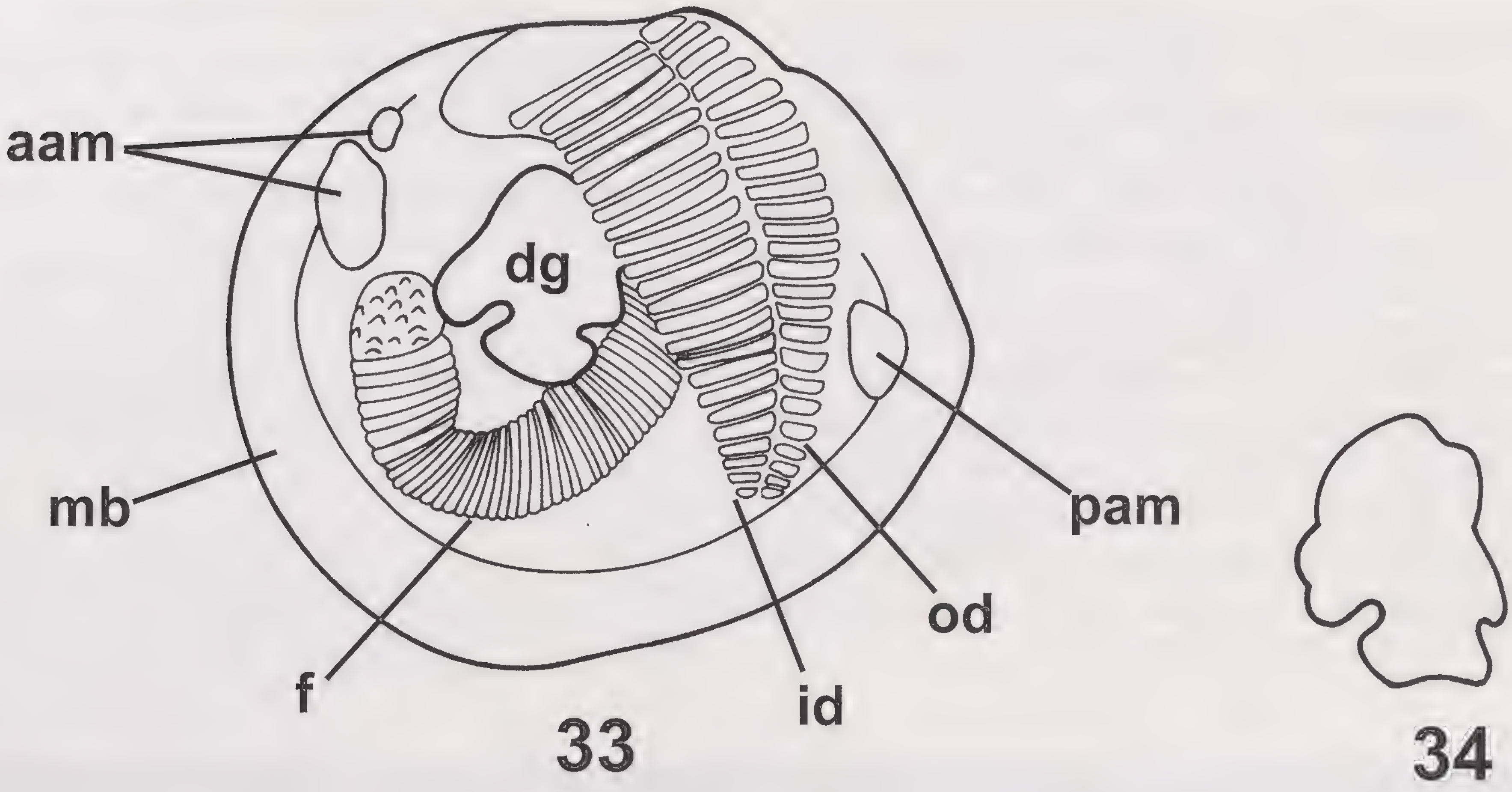
60°52’42”S, 55°44’54”W, 234 m (34 sps, ZMH 997); 60°53’42”S, 55°46’42”W, 320 m (9 sps, ZMH 998); 60°55’00”S, 55°23’48”W, 227 m (22 sps, ZMH 999; 29 sps, ZMH 1000); 62°49’30”S, 60°57’30”W, 184 m (117 sps, ZMH 32956); 61°13’42”S, 55°58’06”W, 146 m (62 sps & 4 vs, ZMB 114668); 61°18’54”S, 58°41’42”W, 491 m (1 sp & 1 v, ZMB 114671); 62°07’12”S, 60°22’48”W (1 sp, ZSM 20013213); 63°04’42”S, 57°31’36”W (4 sps, ZSM 20012832; 12 sps, ZSM 20012834; 1 sp., 20012838); 63°01’06”S, 61°09’06”W (2 sps, ZSM 20012839; 1 sp, ZSM 20012849; 5 sps, 20012842); 62°00’05”S, 60°19’19”W (1 sp, ZSM 20012844); 63°02’18”S, 59°10’24”W (1 sp, ZSM 20012836); 62°55’00”S, 57°39’30”W (9 sps, ZSM 20012829); 62°30’00”S, 56°55’48”W (12 sps, ZSM 20012830); 62°07’12”S, 60°22’48”W (2 sps, ZSM 20012833); King George Island [62°13’S, 58°40’W], 15–150 m (77 sps, ZMH 288; 5 sps, ZMH 304; 30 sps, ZMH 305); Fildes Strait [62°14’S, 59°00’W], 15–20 m (1 sp, ZMB 114661); Maxwell Bay [62°10–19’S, 58°35–58’ W], 20–100 m (4 sps, ZMB 116662; 11 sps, ZMB 116663; 3 sps, ZMB 116665). South Orkney Islands: 61°10’49”S, 45°42’43”W, 322 m (44 sps, MLP 12705); 60°34’54”S, 44°17’00”W, 240 m (21 sps, ZMH 289); 60°51’18”S, 44°12’00”W, 178 m (48 sps, ZMH 290); 60°47’12”S, 45°44’24”W, 237 m (96 sps, ZMH 292); 61°03’12”S, 45°24’36”W, 248 m (13 sps, ZMH 293); 61°07’30”S, 46°31’30”W, 289 m (59 sps, ZMH 294); 60°55’00”S, 46°47’48”W, 320 m (63 sps, ZMH 295); 60°25’06”S, 45°39’30”W, 285 m (28 sps, ZMH 296); 60°23’06”S, 46°20’24”W, 265 m (144 sps, ZMH 297); 60°23’06”S, 46°44’00”W, 280 m (60 sps, ZMH 298; 13 sps, ZMH 299). South Sandwich Islands: 57°40’33”S, 26°25’08”W, 278–308 m (11 sps, MLP 12706); 59°54’58”S, 32°28’19”W, 518–521 m (13 sps & 2 vs, MLP 12707); 57°40’21”S, 26°27’53”W, 475–587 m (2 sps, MLP 12708); 57°40’46”S, 26°24’54”W, 263–270 m (1 sp, MLP 12709). South Georgia Islands: 54°27’S, 35°40’W, 236–239 m (14 sps & 1 v, MLP 12710); 54°27’S, 35°41’W, 249–256 m (24 sps, MLP 12711); 54°30’S, 35°50’W, 94 m (17 sps, MLP 12711). Shag Rocks: 53°23’48”S, 42°42’01”W, 313 m (1 sp & 1 v, MLP 12713). Burdwood Bank: 54°30’13”S, 56°08’12”W, 286–290 m (7 sps & 6 vs, MLP 12714); 54°01’21”S, 62°01’19”W, 272 m (1 v, MLP 12702). Beagle Channel: 54°53’06”S, 69°30’30”W, 62 m (2 sps, ZMH 33047); 54°56’00”S, 69°14’18”W, 208 m (1 sp & 1 v, ZMH 19764). Strait of Magellan: 53°28’48”S, 70°21’54”W, 92 m (7 sps, ZMH 19757). Drake Passage: 55°28’48”S, 66°03’24”W, 1,279 m (42 sps, ZMB 103642); 55°27’24”S, 66°06’18”W, 780 m (1 sp, ZMB 103687).

Description

Shell small (maximum observed H = 2.3 mm), subovate, inflated (W/H = 0.66 ± 0.04, n = 24), delicate, inequilateral. Anterior end widely projecting; posterior end short, angulated (Figs. 22–27). Anterior and posterior ends frequently incrusted with ferruginous material. Anterior part of dorsal margin nearly straight, horizontal to slightly sloping downwards, forming slight



FIGS. 22–32. *Thyasira debilis*. FIG. 22: Holotype (ZMB 63112); FIG. 23: Specimen from 59°54'58"S, 32°28'19"W, 518–521 m (MLP 12707); FIG. 24: Specimen from 54°30'S, 35°50'W, 94 m (MLP 12711); FIGS. 25, 28, 29: Specimens from 70°52'52"S, 98°26'07"W, 425 m (MLP 12703); FIGS. 26, 27, 30, 31: Specimens from 61°23'27"S, 55°26'49"W, 285–288 m (MLP 12704); FIG. 32: Specimen from 55°28'48"S, 66°03'24"W (ZMB 103642); FIGS. 22–24: Outer view right valve; FIGS. 25–27: Outer view left valve. FIGS. 28, 29. Inner view left valve. FIG. 28: General view; FIG. 29: Detail of hinge; FIGS. 30, 31. Inner view right valve. FIG. 30: General view; FIG. 31: Detail of hinge; FIG. 32: Protoconch. Scale bars FIGS. 22–24, 26–28, 30 = 1 mm; FIGS. 25, 29, 31 = 200 µm; FIG. 32 = 50 µm.



FIGS. 33, 34. *Thyasira debilis*. FIG. 33: Anatomy of a decalcified specimen; FIG. 34: Detail of digestive gland. aam: anterior adductor muscle; dg: digestive gland; f: foot; id: inner demibranch; mb: mantle border; od: outer demibranch; pam: posterior adductor muscle.

angle with anterior margin (Figs. 27, 28, 30). Anterior margin widely curved; ventral margin evenly rounded (Figs. 22–27). Posterior part of dorsal margin sloping rapidly and nearly straight from beak. Posterior area of shell poorly discernible from central area. Primary sulcus only defined by slightly flattening in posterior margin; auricle variably projecting (Figs. 22–27). First and second posterior folds obsolete. Lunule ill defined. Beaks small, full, posteriorly located. Protoconch ovate, of about 135 µm in diameter, smooth (Fig. 32). Shell surface whitish, brilliant, sculptured with low growth lines. Periostracum thin, cream. Hinge plate narrow, with variably developed anterior pseudocardinal tubercle in right valve, and corresponding depression in left valve (Figs. 28–30). Ligament short, located into posterior ligamental groove, not visible externally.

Anatomy: Mantle margin wide, largely free for about 3/4 of its length. Only one siphonal oppening present; presiphonal suture short. Transverse section of anterior adductor muscle large, formed by 2–3 well-defined areas; transverse section of posterior adductor muscle small, about one-third anterior muscle (Fig. 33). Both inner and outer demibranchs, present, formed by 25–32 filaments in adult specimens; outer demibranch about half height of inner one. Ascending and descending lamellae of inner demibranch equally developed; descending lamella of outer demibranch very short, approximately 1/6 high of ascending lamella. Labial palp smooth. Digestive gland small, forming three main lobes: anterior slightly trilobed, ventral bilobed, and posterior of undulating outline (Fig. 34). Foot long, stout, vermiform at stem, with well-separated distal bulb differentiated into two main areas: proximal with corrugated surface (as foot stem) and distal with warts (Fig. 33). Heel not demarked.

Remarks

Thyasira debilis has frequently been confused in the literature with *Axinus bongraini*. Nicol (1966) first reported *T. debilis* in the synonymy of *Axinus bongraini* (as *Genaxinus*), a conclusion subsequently followed by Hain (1990). Dell (1990) also inferred that "the single specimen of a thyasirid which Lamy described as *bongraini* from off the Antarctic Peninsula should represent the only common thyasirid known from this area, i.e. "*debilis*"". *Axinus bongraini* is here regarded as a *nomen dubium*; however, the original description by Lamy (1911a) and the redescription and figure the author subsequently provided (Lamy, 1911b) show that *Axinus bongraini* clearly differs from *Thyasira debilis* in having a high shell, with triangular shell outline, a long anterior part of dorsal margin which slopes downwards quickly, and a widely rounded ventral margin. These differences clearly preclude these species from being synonyms.

It should be noted that Dell's (1990) report of *Thyasira debilis* as the only thyasirid occuring in the vicinity of the Antarctic Peninsula, is incorrect. In this area also occurs *Parathyasira dearborni*. These two species are clearly different: *P. dearborni* is characterized by a rhomboidal, higher than long shell outline, with subcentrally located beaks, and edentulous hinge, while *T. debilis* has a subovate, longer than high shell outline, posterior beaks, and hinge with a pseudocardinal tubercle. The morphological characters described above also allow the separation of *Thyasira debilis* from any other thyasirid species occurring in the area. Anatomically, this species is characterized by having a small digestive gland, and extremely short ascending lamellae in the outer demibranch.

Parathyasira Iredale, 1930

Type Species: *Parathyasira resupina* Iredale, 1930 (by original designation)

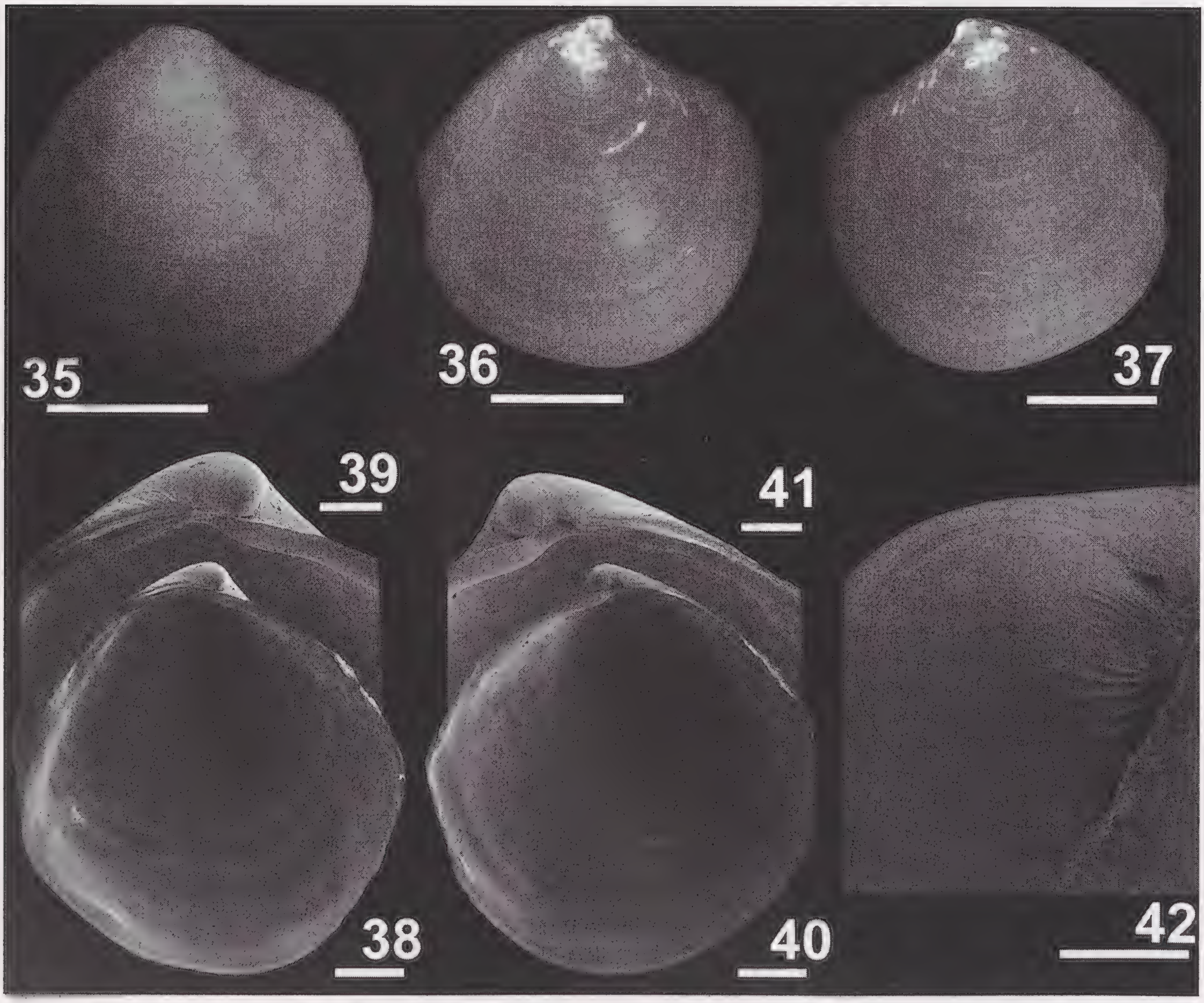
Parathyasira magellanica (Dall, 1901), new combination

Figures 1, 35–45

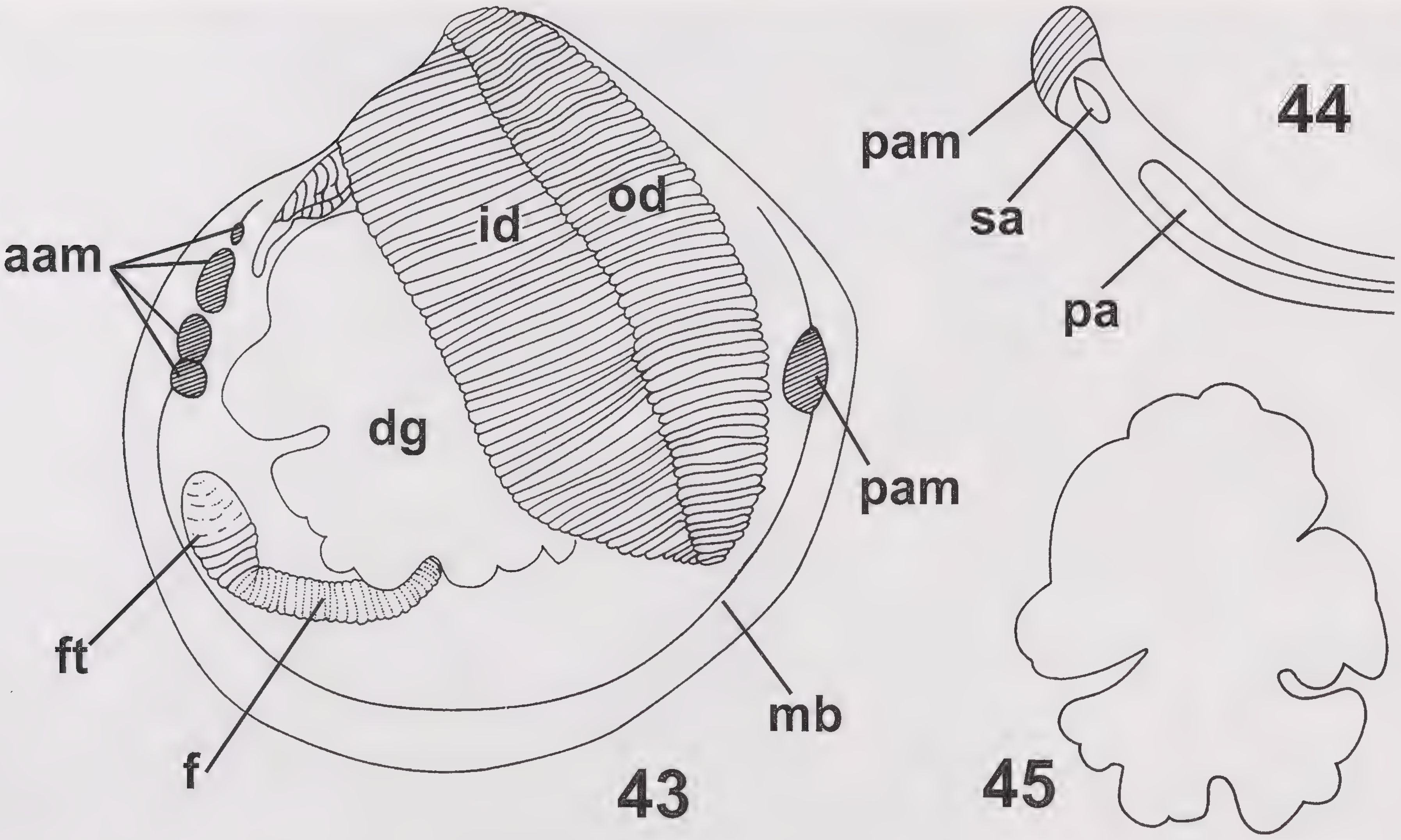
Thyasira magellanica Dall, 1901: 819, pl. 42, fig. 6; Carcelles, 1950: 78, pl. 4, fig. 77.

Type Locality

West coast of Patagonia, 194 fathoms [355 m].



FIGS. 35–42. *Parathyasira magellanica*. FIG. 35: Holotype (USNM 122745); FIGS. 36, 37, 42: Specimens from 54°55'24"S, 69°19'48"W, 272 m (ZMH 19762); FIGS. 38–41: Specimen from 54°49'S, 68°14'W, 126–135 m (MLP 12969); FIGS. 35, 36: Outer view right valve; FIG. 37: Outer view left valve; FIGS. 38, 39: Inner view left valve. FIG. 38: General view; FIG. 39: Detail of hinge; FIGS. 40, 41. Inner view, right valve. FIG. 40: General view; FIG. 41: Detail of hinge; FIG. 42: Protoconch. Scale bars FIGS. 35–37 = 2 mm; FIGS. 38, 40 = 500 μm; FIGS. 39, 41 = 200 μm; FIG. 42 = 50 μm.



FIGS. 43–45. *Parathyasira magellanica*. FIG. 43: Anatomy of a decalcified specimen; FIG. 44: Detail of posterior part of mantle margin; FIG. 45: Detail of digestive gland. aam: anterior adductor muscle; dg: digestive gland; f: foot; ft: foot tip; id: inner demibranch; mb: mantle border; od: outer demibranch; pa: pedal aperture; pam: posterior adductor muscle; sa: siphonal aperture.

Material Examined (Fig. 1)

Holotype (USNM 122745). Beagle Channel: 54°53’06”S, 69°30’30”W, 62 m (3 sps, ZMH 19760); 54°55’24”S, 69°19’48”W, 272 m (1 sp, ZMH 19761; 8 sps & 1 v, ZMH 19762; 5 sps & 1 v, ZMH 19763); 54°54’54”S, 68°39’06”W, 310 m (1 sp, ZMH 19765); 54°49’S, 68°14’W, 126–135 m (1 sp, MLP 12969); 54°49’S, 68°13’W, 138–144 m (1 sp, MLP 12697). Magellan Strait: 53°43’18”S, 70°56’06”W, 335 m (1 sp & 4 vs, ZMH 19754); 53°42’48”S, 70°57’30”W, 51 m (2 sps, ZMH 19755); 53°32’54”S, 70°39’12”W, 462 m (2 sps, ZMH 19758); 53°42’42”S, 70°50’06”W, 522 m (3 sps, ZMH 19759).

Description

Shell medium in size (maximum observed H = 7.5 mm), subquadrate, approximately equilateral, somewhat inflated (W/H = 0.57 ± 0.05, n = 15), delicate, translucent. Anterior end slightly horizontally projecting (Figs. 35–37). Anterior part of dorsal margin sloping downwards gradually, almost straight, with marked angle at junction with anterior margin, which is almost vertical, evenly rounded or angled (Figs. 35–38, 40). Ventral margin markedly curved, somewhat anteriorly expanded, insensibly connected with anterior margin. Posterior area of shell clearly distinguished from central area (Figs. 36, 37). First posterior fold absent; second posterior fold low, rounded. Posterior sulcus narrow, shallow, resulting in a flat or weakly sinuate posterior margin (Figs. 35–38, 40). Auricle absent. Submarginal sulcus long, deeply incised, almost vertical. Lunule small, narrow. Beaks pointed, subcentrally located, anteriorly directed. Protoconch outline ovate, about 120 µm in diameter; initial part sculptured with 5–7 lamellated folds, sometimes bifurcated at base, symmetrically distributed with respect to main central axis (Fig. 42). Shell surface shiny, with fine, low growth lines, increasing in solidness towards ventral margin. Periostracum thin, translucent. Hinge plate narrow, edentulous, with narrow posterior ligamental groove (Figs. 38–40); ligament internal, externally visible, representing one-half submarginal sulcus length.

Anatomy: Mantle margin largely free, for about 3/4 of its length. Small (exhalant) posterior aperture present; presiphonal suture short (Fig. 44). Transverse section of anterior adductor muscle elongated, showing in larger specimens usually four well-defined areas; transverse section of

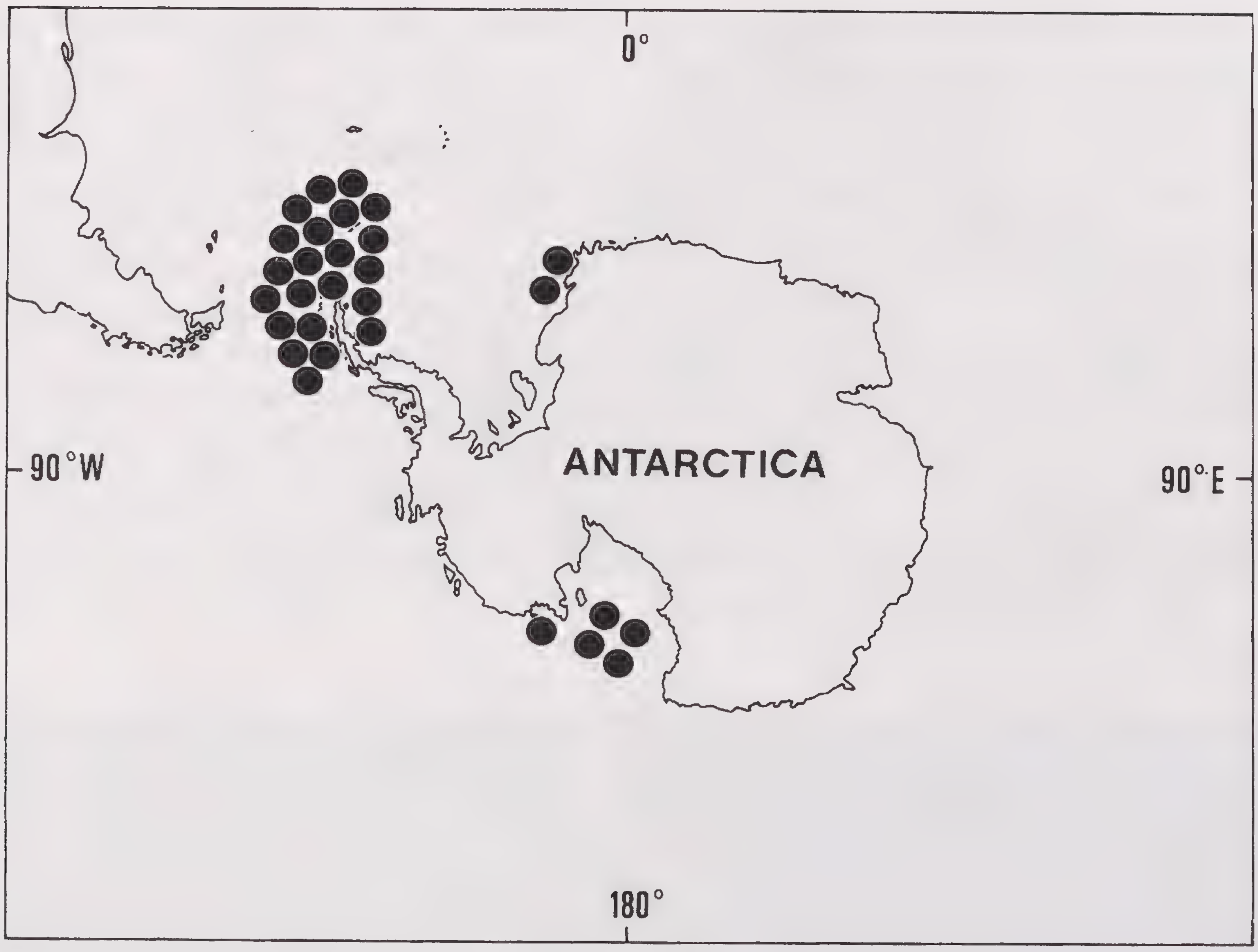


FIG. 46. Known distribution of *Parathyasira dearborni.*

posterior adductor muscle ovate, much smaller than anterior one (Fig. 43). Gills: both inner and outer demibranchs, formed by 55–65 filaments in adult specimens present; outer demibranch about half high of inner (Fig. 43). Ascending and descending lamellae of inner demibranch similar in size; descending lamella of outer demibranch one-half length of ascending lamella. Labial palp with 3–4 prominent sorting ridges. Digestive gland with four main lobes: large anterior auricular lobe, two ventral lobes, and small posterior lobe (Fig. 45). Foot vermiform, corrugated at stem and base of bulbous tip; distal bulbous tip with wart-like surface (Fig. 43); heel not demarcated.

Remarks

Parathyasira magellanica closely resembles *Parathyasira dearborni*, from which it may be separated by the shorter anterior end, projecting horizontally (instead of obliquely); these characteristics result in a wider and proportionally lower shell outline. These species also differ in anatomy: *P. magellanica* has only one siphonal opening instead of two as in *P. dearborni*. Oliver & Sellanes (2005) suggested that *Parathyasira magellanica* may be a juvenile of *Thyasira falklandica*. This opinion is not shared in the present study: *T. falklandica* has a larger and more elongated shell, the anterior end more markedly pointed, and the beaks lower and more rounded. Furthermore, *P. magellanica* has a less solid shell.

Soot Ryen (1951) pointed on the similarities of *Parathyasira magellanica* and *Thyasira* (?) *bongraini*, suggesting that both names may be synonyms. However, the figure of *T. bongraini* by Lamy (1911b), reproduced here as Figure 68, shows a longer anterior part of dorsal margin, sloping downwards more abruptly than in *P. magellanica*, and the anterior margin forming a wide curve with the ventral margin. In the present study, *T. bongraini* is regarded as a *nomen dubium*.

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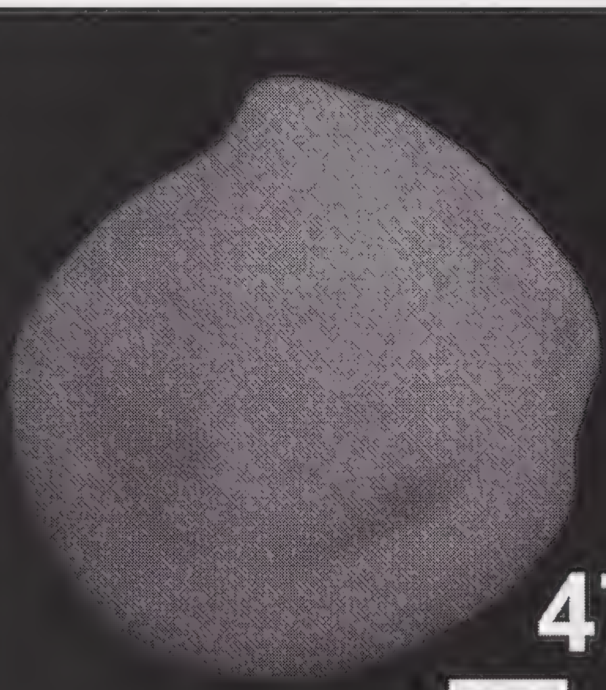
FIGS. 47–59. *Parathyasira dearborni*. FIG. 47: Holotype (USNM 653099); FIG. 48: Specimen from 62°49'30"S, 60°57'30"W, 184 m (ZMH 33053); FIG. 49: Specimen from 77°05'S, 177°12'E (NIWA 45842); FIGS. 50, 56: Specimen from 74°46'24"S, 178°23'24"W (NHM 1964752); FIG. 51: Specimen from 77°27'S, 172°22'E (NIWA 45841); FIG. 52: Specimen from 64°50'35"S, 62°57'55"W (MLP 12699); FIGS. 53–55, 57–59: Specimens from 60°59'11"S, 43°27'25"W (MLP 12701); FIGS. 47–52: Outer view left valve; FIGS. 53, 54: Outer view right valve; FIG. 55: Inner view right valve; FIG. 56: Detail of shell sculpture; FIGS. 57, 58. Detail of hinge. FIG. 57: Left valve; FIG. 58: Right valve; FIG. 59: Protoconch. Scale bars FIGS. 47–55 = 1 mm; FIG. 56 = 100 µm; FIGS. 57, 58 = 500 µm; FIG. 59 = 50 µm.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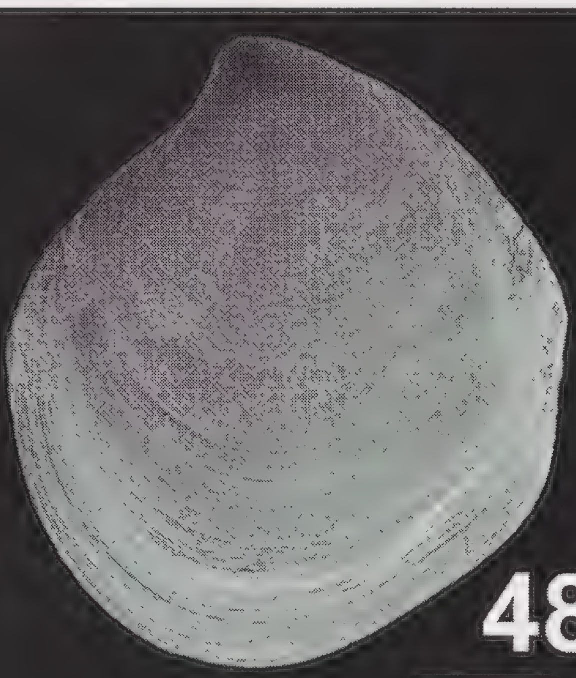
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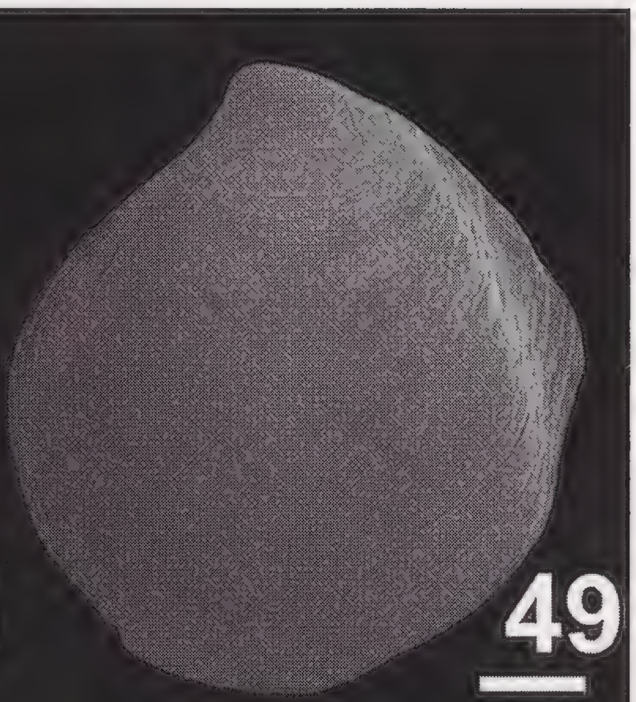
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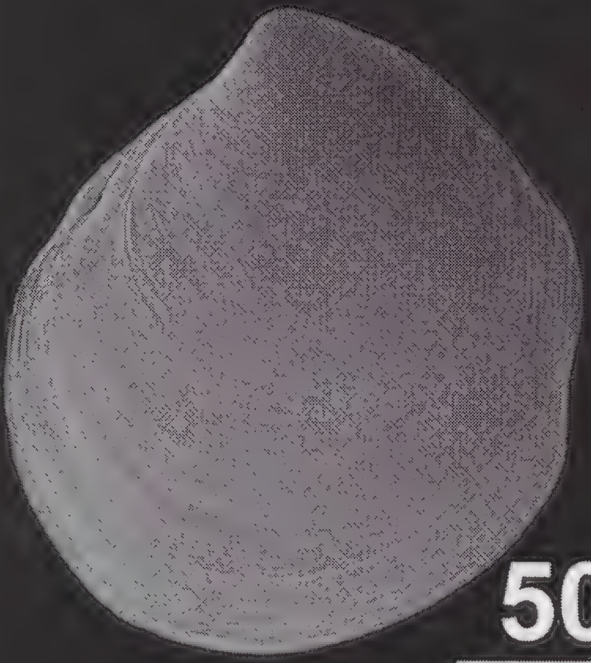
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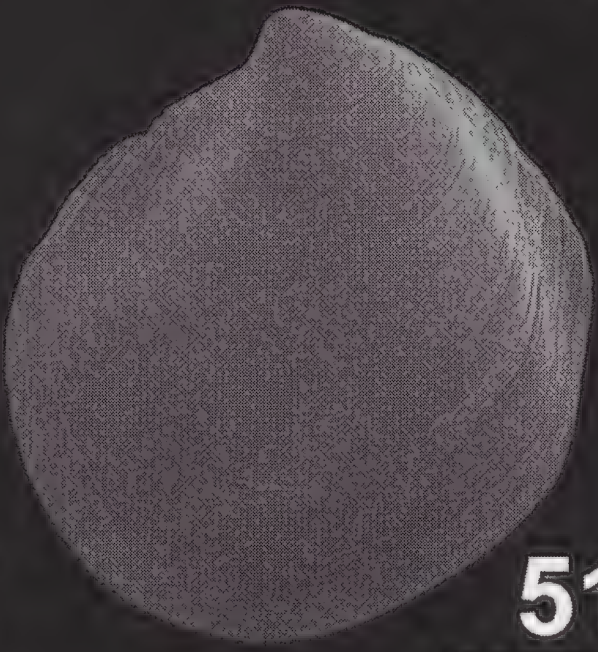
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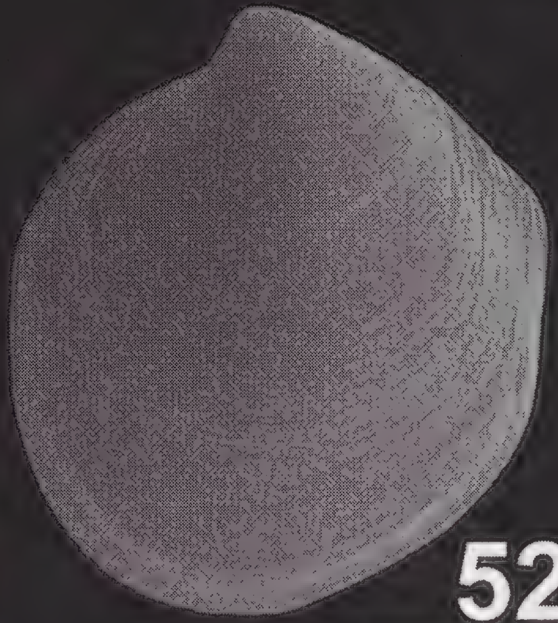
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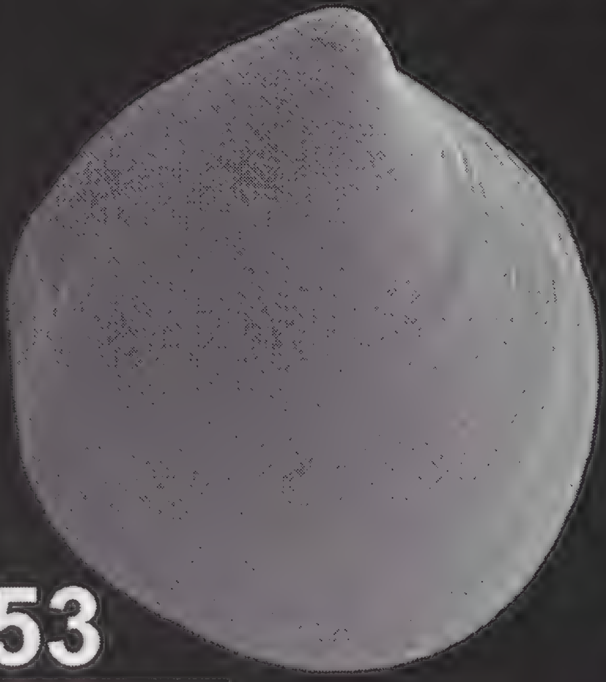
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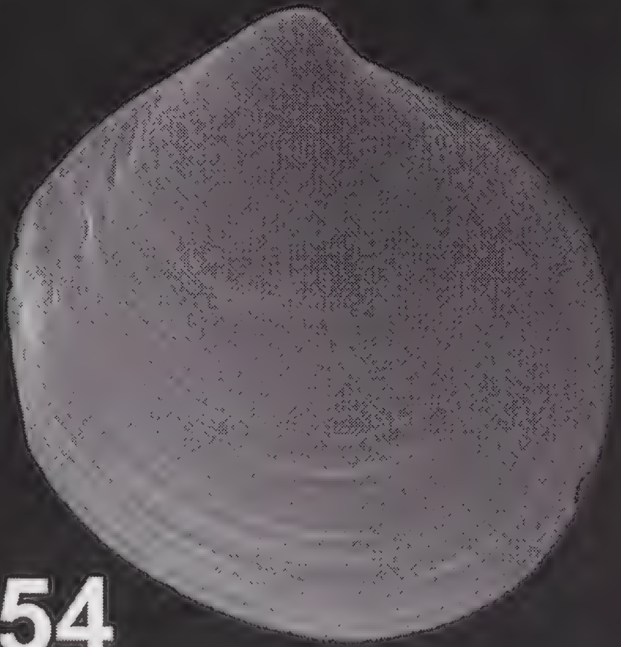
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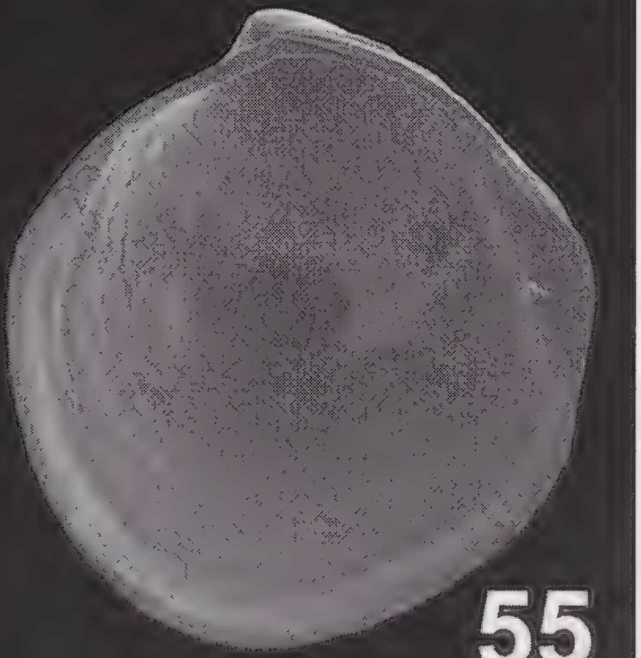
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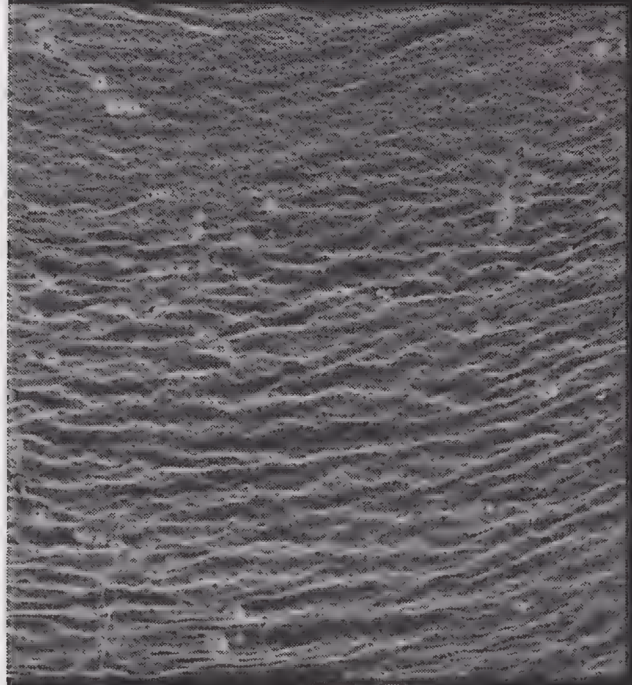
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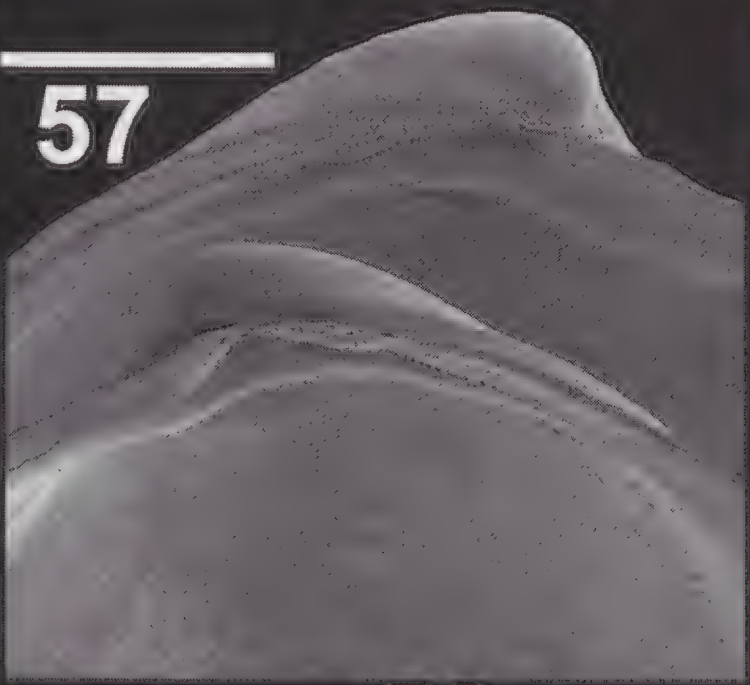
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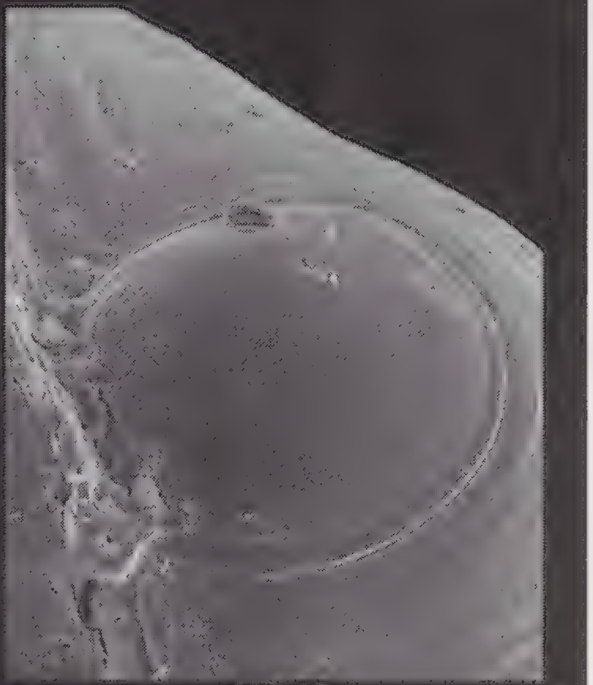
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Parathyasira dearborni (Nicol, 1965), new combination

Figures 46–62

Thyasira bongraini: Mühlenhardt Siegel, 1989: 162 [in part], pl. 3, fig. 24 (*non* Lamy, 1911).

Thyasira cf. *bongraini*: Dell, 1964: 207, fig. 4, # 10, 11.

Thyasira dearborni Nicol, 1965: 79, pl. 8, figs. 1, 2; Dell, 1990: 56, figs. 91, 92.

Type Locality

73°46.7’S, 169°09’E, off Coulman Island [Ross Sea], 836 m.

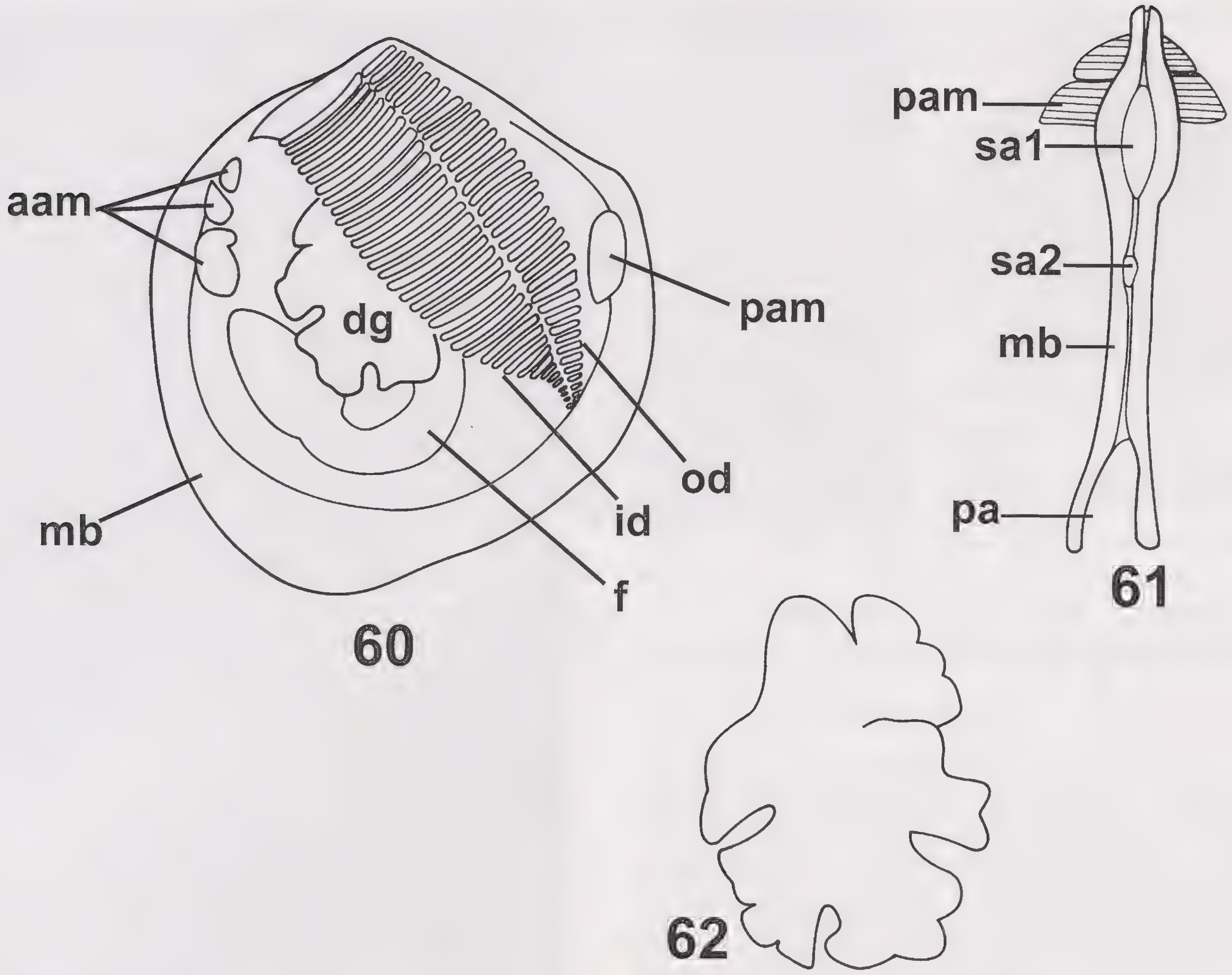
Material Examined (Fig. 46)

Holotype (USNM 653099). Ross Sea: 74°46’24”S, 178°23’24”W, 351 m (2 sps & 2 vs, NHM 1964752); 77°27’S, 172°22’E, 752 m (4vs, NIWA 45841); 75°17’S, 172°20’E, 534–549 m (2 sps, NIWA 45843); 77°05’S, 177°12’E, 362 m (4 vs, NIWA 45842). Antarctic Peninsula: 63°04’42”S, 57°31’36”W (6 sps, ZSM 20012849); 64°50’35”S, 62°57’55”W, 294 m (1 sp, MLP 12699); 63°11’30’S, 58°47’00”W, 93 m (11 sps, ZMH 32975); 66°31’12”S, 68°25’18”W, 464 m (7 sps, ZMH 32960); 65°23’36”S, 66°10’06”W, 420 m (13 sps, ZMH 356); 66°32’06”S, 68°30’06”W, 500 m (4 sps, ZMH 361); 67°16’06”S, 69°21’36”W, 420 m (4 sps, ZMH 362; 5 sps, ZMH 363). Weddell Sea: 71°06’16”S, 12°50’28”W (1 sps, ZSM 20012850); 70°50’36”S, 10°35’24”W (1 sps, ZSM 20012852). South Shetland Islands: 62°07’12”S, 60°22’48”W (2 sps, ZSM 20012850); 61°15’00’S, 55°00’00’W, 120 m (2 sps, ZMH 32974); 62°05’18”S, 57°39’00”W, 265 m (8 sps, ZMH 32963; 5 sps, ZMH 283); 62°59’S, 57°05’W, 68 m (1 sp, ZMH 32965); 62°49’30”S, 60°57’30”W, 184 m (8 sps, ZMH 32957; 2 sps, ZMH 32958); 64°07’36”S, 65°13’06”W, 546 m (1 sp, ZMH 32959); 61°01’18”S, 56°00’24”W, 339 m (2 sps, ZMH 32962); 61°00’42”S, 55°02’24”W, 247 m (3 sps, ZMH 1001); 62°49’30”S, 60°57’30”W, 184 m (1 sp, ZMH 32983; 4 sps, ZMH 32984); 60°57’06”S, 55°55’12”W, 246 m (1 sp, ZMH 329); 61°15’00”S, 55°00’00”W, 120 m (2 sps, ZMH 300); 61°13’48”S, 54°39’12”W, 280 m (1 sp, ZMH 32976; 11 sps, ZMH 32977); 61°03’54”S, 55°58’48”W, 262 m (1 sp, ZMH 32978); 62°46’36”S, 60°54’48”W, 140 m (2 sps, ZMH 32980); 63°16’48”S, 63°44’30”W, 354 m (3 sps, ZMH 32981; 33 sps, ZMH 32982); 62°49’30”S, 60°57’30”W, 184 m (9 sps, ZMH 32986); 62°49’30”S, 60°57’30”W, 184 m (2 sps, ZMH 33053; 5 sps, ZMH 33054); 64°07’36”S, 65°13’06”W, 546 m (3 sps, ZMH 33055); 62°18’54”S, 58°41’42”W, 491 m (1 sp & 1 v, ZMB 114672); Maxwell Bay, King George Island [62°10–19’S, 58°35–58’ W] (5 sps, ZMH 303; 1 sp, ZMB 114664; 1 sp, ZMB 114667). South Orkney Islands: 61°10’48”S, 45°42’43”W, 322 m (1 v, MLP 12700); 60°59’11”S, 43°27’25”W, 402 m (82 sps, MLP 12701); 60°46’36”S, 45°21’54”W, 127 m (6 sps, ZMH 291); 60°51’18”W, 44°12’00”W, 178 m (3 sps, ZMH 32967); 60°47’12”S, 45°44’24”W, 237 m (9 sps, ZMH 32968); 61°07’30”S, 46°31’30”W, 289 m (12 sps, ZMH 32969); 60°55’00”S, 46°46’48”W, 320 m (1 sp, ZMH 32970); 60°25’06”S, 45°39’30”W, 285 m (3 sps, ZMH 32971); 60°23’06”S, 46°20’24”W, 265 m (10 sps, ZMH 32972); 60°23’06”S, 46°40’00”W, 280 m (4 sps, ZMH 32973).

Description

Shell medium in size (maximum H = 6 mm), inflated (W/H = 0.63 ± 0.03, n = 17); anterior end obliquely projecting, particularly in large specimens, resulting in markedly rhomboidal outline (Figs. 47–55). Anterior part of dorsal margin long nearly straight, sloping downwards abruptly, forming weak angle with anterior margin. Anterior margin widely curved, continuous with anterior part of ventral margin; posterior part of ventral margin tending to be parallel to anterior part of dorsal margin (Figs. 47–55). Posterior area of shell flat or with very shallow sinus (Figs. 47–55). Auricle and first posterior fold absent; second posterior fold rounded and weak. Submarginal sulcus long, deeply incised, with almost vertical margins. Beaks rounded, inflated, subcentrally located, anteriorly directed. Protoconch ovate, about 115 µm diameter, initial part sculptured with 14 strong folds, bifurcated at base, symmetrically distributed with respect to main central axis (Fig. 59). Lunule not well defined. Shell surface whitish, dull, sculptured with commarginal ribs composed by microscopic irregular corrugations and pustules (Fig. 56). Periostracum thin, translucent. Ferruginous material sometimes incrusted anterior and posteriorly. Hinge plate narrow, edentulous, with narrow posterior ligamental groove (Figs. 57, 58). Ligament half length of submarginal sulcus, internal but externally visible.

Anatomy: Mantle margin free anteriorly, with long posterior presiphonal suture (1/3



FIGS. 60–62. *Parathyasira dearborni.* FIG. 60: Anatomy of a decalcified specimen; FIG. 61: Detail of posterior part of mantle margin; FIG. 62: Detail of digestive gland. aam: anterior adductor muscle; dg: digestive gland; f: foot; id: inner demibranch; mb: mantle border; od: outer demibranch; pa: pedal aperture; pam: posterior adductor muscle; sa1, sa2: siphonal apertures.

of total mantle margin length), and two small posterior openings (Fig. 61). Mantle margin greatly thickened along pedal aperture. Section of anterior adductor muscle long, formed by three well-differentiated areas in adult specimens, narrowing dorsally. Transverse section of posterior adductor muscle ovate, one-third anterior muscle (Fig. 60). Inner and outer demibranchs present, comprising 43–50 filaments in adult specimens; outer demibranch about one-half height of inner demibranch (Fig. 60). Ascending and descending lamellae of inner demibranch similar in size; descending lamellae of outer demibranch one-half length of ascending demibranch. Labial palp with prominent sorting ridges. Foot long, slender, vermiform at stem, bearing sharp tip (Fig. 60); heel not demarked. Digestive gland with five main lobes: large, auriculiform anterodorsal lobe with central depression, two ventral lobes, and two posterior lobes; ventral and posterior lobes smaller than anterior lobe, with shallow, rounded subdivisions (Fig. 62).

Remarks

Parathyasira dearborni resembles *P. magellanica*. However, both species are easily separated based on shell morphology and anatomy. In *Parathyasira dearborni* the anterior part of dorsal margin is longer, sloping downwards more abruptly, and straighter than in *P. magellanica*, and the anterior end more obliquely projecting, resulting in a higher shell with a more rhomboidal shell outline. On the other hand, *P. magellanica* has subquadrate shell, and the anterior end is not obliquely projecting. In addition, while *P. bongraini* has two siphonal openings, *P. magellanica* has only one.

Mühlenhard Siegel (1989) reported as *Thyasira bongraini* specimens from South Shetland Islands, South Orkney Islands, and the Antarctic Peninsula; the re-examination of this material (ZMH) showed that some of these records correspond to *T. dearborni*. Re-examination of the specimens from the Ross Sea reported by Dell (1964) as *Thyasira cf. bongraini* (NHM 1964752) reveals that they actually correspond to *T. dearborni*.

SPECIES PREVIOUSLY REFERRED AS *THYASIRA*

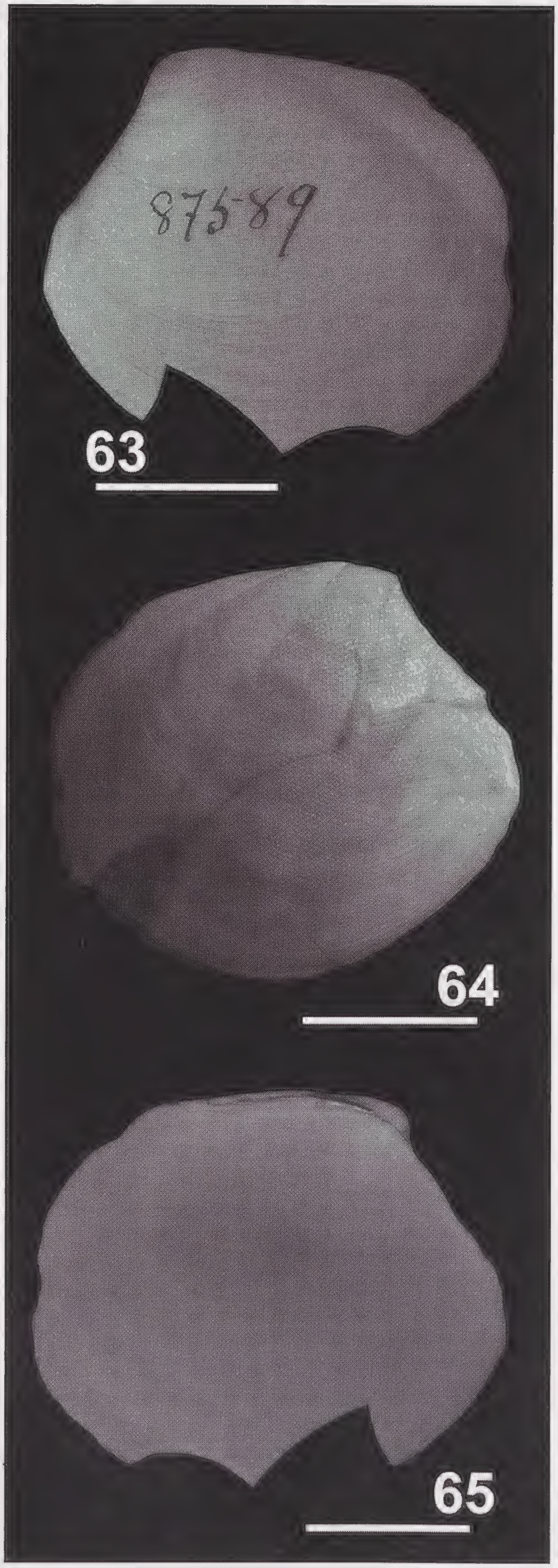
Conchocele fuegiensis (Dall, 1890), new combination (Figs. 63–65). This species, described from 53°06'00'S, 70°40'30"W, Magellan Strait, 142 m, was originally placed under the genus *Cryptodon* Turton, 1827, and reported latter under its senior synonym, *Thyasira*, by Dall (1901). In the present study, the species is reallocated into *Conchocele* Gabb, 1866, based on its large size (holotype 26.3 mm L), elongate shell outline with anteriorly displaced beaks, deep submarginal sulcus, and presence of a marginal sinus; these characters were considered as diagnostic for *Conchocele* by Coan et al. (2000) and Oliver & Sellanes (2005).

Conchocele fuegiensis resembles *Conchocele bisecta* (Conrad, 1849) from the northern Pacific Ocean but differs by having a bisinuate posterior margin. Furthermore, in *C. bisecta* the beaks are at anterior end, and the anterior margin slopes almost vertically, while in *C. fuegiensis* the beaks are subterminal and the anterior margin is slightly projecting. Oliver & Sellanes (2005) reported specimens from off Concepción (Chile) as *Conchocele* sp. These specimens differ from *C. fuegiensis* by having a concave anterior margin and single posterior sinus.

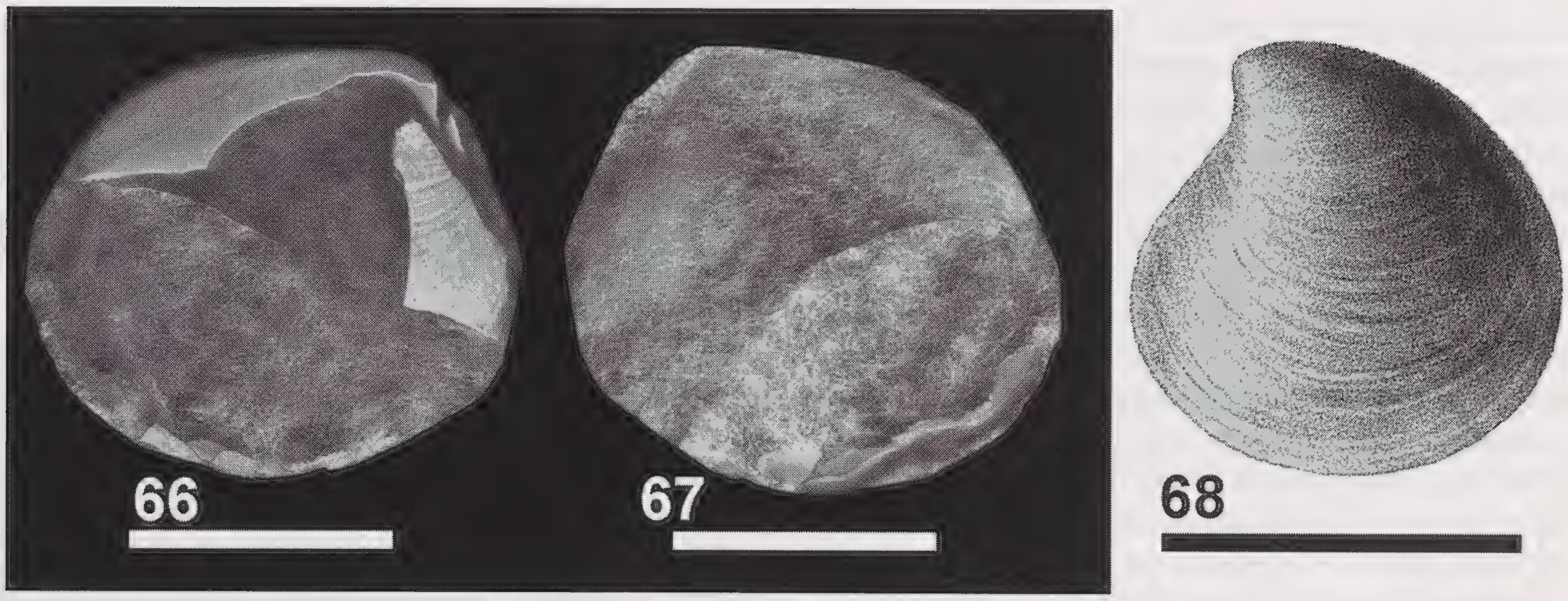
NOMEN DUBIA

Loripes pertenuis Smith E. A., 1881 (Figs. 66, 67) was described from the Magellan Strait. Soot Ryen (1959) suggested that the species might be placed under *Thyasira* and be a synonym of *T. magellanica* (*Parathyasira* in this study). However, the original description and figure of *Loripes pertenuis* show clearly an obliquely subcircular shell outline, with anteriorly displaced beaks and bisinuate posterior margin; this set of characters do not agree with those of *P. magellanica*, fitting instead with the concept of *Conchocele fuegiensis*; however, the rather poor state of preservation of the type specimen of *Loripes pertenuis* prevent us to confirm the relationship of these two species.

Axinus bongraini Lamy, 1911, was described from Petermann Island [65°11'S, 64°10'W, west of the Antarctic Peninsula]. The single type specimen (MNHN) is not informative because it is now completely fragmented; the original description of the species is poor and does not provide any anatomical information. The subsequent figure by Lamy (1911b) disregards relevant morphological details such as the morphology of the pos-



FIGS. 63–65. *Conchocele fuegiensis*. Holotype (USNM 87589). FIG. 63: Outer view left valve; FIG. 64: Outer view right valve; FIG. 65: Inner view left valve. Scale bars = 10 mm.



FIGS. 66–68. Species remaining as *nomen dubia*. FIGS. 66, 67: *Loripes pertenuis*, holotype (NHM 1879.10.15.72); FIG. 66: Right valve; FIG. 67: Left "valve"; FIG. 68: *Axinus bongraini*, original figure by Lamy (1911b, pl. 1, fig. 17). Scale bars = 5 mm.

terior area (i.e., the presence of auricle, sulcus, and sinus) and hinge plate. Due to this, *Axinus bongraini* is regarded as a *nomen dubium*. Dell (1990) pointed that "the identity of Lamy's [*Axinus*] *bongraini* cannot ... be readily determined"; despite this, the author suggested it might be a synonym of *Parathyasira dearborni*. However, none of the specimens of *Parathyasira dearborni* studied here showed a ventral margin so evenly rounded as it was figured by Lamy (1911b), for which Dell's opinion is not shared.

Nicol (1966) and Hain (1990) reported *Axinus bongraini* (under *Genaxinus*) as a synonym of *Thyasira debilis*. However, the smaller shell, with ovate shell outline, strongly projecting anterior end, and nearly undifferentiated posterior area of *T. debilis*, clearly preclude these two species being synonyms.

The specimens reported by Dell (1964) as *Thyasira* cf. *bongraini* (NHM 1964752) and those reported by Mühlenhardt Siegel (1989) as *Thyasira bongraini* (ZMH, in part) are here reassigned to *Parathyasira dearborni*.

Egorova (1982) assigned to *Thyasira bongraini* specimens from Davis Sea (East Antarctica). However, the figures provided (Egorova, 1982: figs. 311–313) show a wide auricle and a prominently differentiated lunule, two characters not reported in the original description nor figured by Lamy (1911b) for the species.

DISCUSSION

The identity of the Magellanic and Antarctic thyasirids has been confusing. Several facts have contributed to this situation, among them, some species names, such as *Thyasira bongraini*, were applied erroneously to referrs to *Parathyasira dearborni* or *Thyasira debilis*; in other cases, species were wrongly assigned to the genera *Genaxinus*, in the case of *Thyasira debilis*, or *Thyasira* for *Conchocele fuegiensis*. This situation seems to have originated from the vague, poorly detailed, original descriptions, as well as in from the few specimens upon which several species were based. The present study has addressed this problem by revising all species reported for the area, with descriptions and illustrations of all traceable types, as well as numerous additional specimens from the Magellanic and Antarctic waters, giving attention to morphological and anatomical characters, as well as intraspecific variability.

Five thyasirid species previously described from the Magellanic and Antarctic waters are considered valid: *Thyasira debilis*, *T. falklandica*, *Parathyasira magellanica*, *P. dearborni*, and *Conchocele fuegiensis*; a sixth species, ***Thyasira scotiana*** from the Scotia Arc Islands, is here described; and *Loripes pertenuis* and *Axinus bongraini*, the other two species previously assigned to *Thyasira*, are considered *nomen dubia*. These species are known only from the type material, and the state of preservation of these specimens precludes any well-based conclusion on the status of these taxa.

Shell Morphology and Soft Part Anatomy

Taking into account the shell morphology, *Conchocele fuegiensis* appears the most easily distinguished thyasirid in Magellanic and Antarctic waters: its large size, elongate shell outline, anteriorly placed beaks, bisinuate posterior margin, and elongate anterior adductor muscle scar are diagnostic. Other thyasirid species with an elongate (longer than high) shell outline are *Thyasira debilis* and *T. falklandica*. Both of them are smaller than *Conchocele fuegiensis*. Furthermore, in the former the beaks are posteriorly located, the anterior end is more prominently projecting, and the posterior area is only slightly flattened. In *T. falklandica* the beaks are subcentral and there is only one posterior sinus. The remaining three species from the area have subcircular (***Thyasira scotiana***), subquadrate (*Parathyasira magellanica*), or rhomboidal (*P. bongraini*) shell outlines, that is, shells as high as long or higher than long.

The size of the protoconch is similar in *Parathyasira magellanica*, *P. dearborni*, and *Thyasira debilis*, ranging from 115 to 135 μm in diameter, but in ***T. scotiana*** the protoconch is considerably larger (about 210 μm); this character is unknown in *Thyasira falklandica.* Regarding protoconch sculpture, two patterns may be recognized: *Thyasira debilis* has a smooth protoconch, while *Parathyasira magellanica*, *P. dearborni*, and ***T. scotiana*** show a series of prominent radial folds symmetrically arranged along a main central axis (this pattern is being reported for the first time in thyasirids).

All studied species have a narrow hinge plate. *Parathyasira magellanica*, *P. dearborni*, ***Thyasira scotiana***, and *T. falklandica* have no hinge teeth, while *Thyasira debilis* shows a prominent anterior tubercle in the right valve.

In *Parathyasira magellanica*, *P. dearborni*, ***Thyasira scotiana***, and *T. debilis*, the gill is composed by two, inner and outer, demibranchs. In *Parathyasira magellanica* and *P. dearborni*, the ascending lamellae of the outer demibranch are a half the high of descending lamella, while in *Thyasira debilis* the descending lamellae are extremely short.

The digestive gland appears widely laterally expanded in all the species in which anatomy was studied. In *Parathyasira magellanica* and *P. dearborni*, it is large and multilobed; on the other hand, *Thyasira debilis* has a proportionally smaller and poorly lobed digestive gland, a condition resembling that in *T. gouldi* (Philippi, 1845), *T. succisa* (Jeffreys, 1876), and *T. obsoleta* (Verrill & Bush, 1898).

The mantle margin of *Parathyasira magellanica* and *Thyasira debilis*, as well as other species of the genus for which the anatomy is known, is largely free, determining a relatively large pedal aperture and a small posterior exhalant opening. *Parathyasira dearborni* has a comparatively shorter pedal aperture, determined by the presence of a larger presiphonal suture, and two posterior openings.

Generic Assignement of Species

Parathyasira magellanica and *P. dearborni* were formerly reported under *Thyasira*, however, they have a subquadrate to rhomboidal shell outline ("pyriform" or "polygonal" of authors), almost vertical submarginal sulcus with sharp border, flat to almost flat posterior area, and absence of auricle; this set of characters were recognized as diagnostic of *Parathyasira* (Payne & Allen, 1991; Oliver & Killeen, 2002; Oliver & Holmes, 2006). This two species show differences in the number of posterior openings, one in *P. magellanica*, two in *P. dearborni*. The presence of two posterior openings could be viewed as a valuable character for an eventual generic splitting; however, this is not possible at the present state of knowledge of thyasiriids. Anatomical details of the type species of *Parathyasira* are unknown. Payne & Allen (1991) pointed out on the great variation in the degree of mantle margin fusion among the species they referred to as members of *Parathyasira*. The authors reported in some species the occurrence of temporary adhesions of the mantle margin, to form an "inhalant" aperture. This condition clearly differs from that present in *Parathyasira dearborni*, were the apertures are not temporary but permanent (i.e., the mantle margins are fused above and below both apertures).

Thyasira debilis was originally described under the genus *Axinopsis* Sars, 1878. However, this species clearly differs from the type species of *Axinopsida* Keen & Chavan (in Chavan), 1951 (*nomen novum pro Axinopsis* Sars, 1878, *non* Tate, 1868), *A. orbiculata* Sars, 1878, which is characterized by having a suborbicular shell outline, concave lunule margin, and ovate-elongate muscle scars. None of these characters are present in *T. debilis*. Dell (1964), based on the similarities of this species "with a New Zealand group of species classified under *Genaxinus* by Fleming (1950)" [i.e., "*Genaxinus*" *cookianus* Fleming, 1950, and "*Genaxinus*" *otagoensis* (Suter, 1913)], transferred *Thyasira debilis* to *Genaxinus* Iredale, 1930. This generic assignment was subsequently widely used in the Antarctic literature. *Thyasira debilis* is actually similar in shell morphology to the New Zealand species reported by Fleming under *Genaxinus*, but all these species differ strikingly from *Thyasira albigena* Hedley, 1907, the type species of *Genaxinus*. That species is characterized by a higher than long shell, with subcentral beaks, edentulous hinge plate, and large, elongate, similar in size anterior and posterior muscle scars. On the contrary, in the Antarctic and New Zealand species the shell outline is subovate (longer than high), with posterior beaks, the anterior end prominently projecting, the muscle scars are small and inequal, and hinge with a prominent tubercle.

The reallocation of *Axinopsis debilis* Thiele under *Thyasira*, as proposed here, is based on the morphological and anatomical characters of the species, namely, the presence of a shell with elongate anterior adductor scar, ovate posterior adductor scar, vermiform foot, and two demibranchs. These characters were recognized as diagnostic for *Thyasira* by Oliver & Killeen (2002), Oliver & Sellanes (2005), and Oliver & Holmes (2006). However, *T. debilis* lacks the characteristic bisulcated and bisinuated posterior margin, reported by Oliver & Killeen (2002) for *Thyasira* s.s. In addition, *T. debilis* has a relatively small, poorly lobed digestive gland, which is in contrast with the "large and multilobed" gland described by Oliver & Holmes (2006) for the genus. Despite that, the morphology of the digestive gland of *T. debilis* is similar to that present in *Thyasira succisa* (Jeffreys, 1876), a species retained under *Thyasira* in the most recent revisions (Payne & Allen, 1991; Oliver & Killeen, 2002). With that species, *T. debilis* also share the presence of extremely short descending lamellae of outer demibranch, an ovate shell outline, and a cardinal tubercle in hinge. These morphological characters are also present in "*Genaxinus*" *cookianus*, "*Genaxinus*" *otagoensis*, and *Thyasira succisa atlantica* Payne & Allen, 1991, suggesting that all of them might represent a distinct (sub)genus. But as "the current definitions of the genera of the Thyasiridae are poor and inadequately reflect the diversity of shell and anatomy", as stated by Oliver & Holmes (2006), I prefer to be conservative and do not introduce any new generic names.

Thyasira falklandica and ***T. scotiana*** also lack a bisinuated posterior margin, but as the soft part anatomy of the species is also unknown, a discussion on their generic placement is postponed. In the meanwhile, they are retained as *Thyasira* s.l., as was done by Oliver & Holmes (2006) for some species of the Northern Hemisphere.

Distribution of the Species

Among the thyasirid species recognized as valid in the present study, *Thyasira debilis* appears as the most widespread distributed: occurring around Antarctica, from the Ross Sea to the Davis Sea, extending northwards up to Tierra del Fuego in the Magellan Region. The remaining five species seems to have a more restricted geographic distributions, occurring in the Magellanic (*Parathyasira magellanica*, *Thyasira falklandica*, and *Conchocele fuegiensis*) or the Antarctic (*Parathyasira dearborni* and ***Thyasira scotiana***) waters. Within the Magellan Region, *Parathyasira magellanica* and *Conchocele fuegiensis* are known from the Beagle Channel and the Strait of Magellan, extending northward the west coast of Patagonia, to Chiloé Islands (Dall, 1901; Soot Ryen, 1959); and *T. falklandica* from the vecinty of the type locality in the Malvinas Islands. Among the Antarctic species, *P. dearborni* was recorded from the Ross Sea to the Weddell Sea, extending northward up to the southernmost islands of the Scotia Arc; and ***T. scotiana*** is thus far only known from the type locality.

In addition to its wide geographic distributional range, *Thyasira debilis* also exhibits a wide bathymetrical range, occurring from the shallow waters up to 1,279 m depth. The species with the narrowest bathymetric ranges are ***Thyasira scotiana*** and *Thyasira falklandica*. The remaining three species, although restricted to a single biogeographic realm (Antarctic or Magellan Region), all surpass the outer limits of the continental shelf: *T. dearborni* is present to 1,180 m depth (Dell, 1990); *Thyasira magellanica* to 522 m depth (this study); and *Conchocele fuegiensis* to 822 m depth (Dall, 1901). These results seem to indicate that depth itself is not limiting species distribution.

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SYMPATRIC CHARACTER CONVERGENCE BETWEEN TWO SPECIES OF CLOSELY RELATED GENERA OF PHOLADINAE (BIVALVIA: PHOLADIDAE) FROM THE EASTERN COAST OF INDIA

Tanmoy Hari[1]*, Subhendu Bardhan[1] & Debahuti Mukherjee[2]

ABSTRACT

Biological interaction between two ecologically related species in sympatry results mostly as character divergence, which is also known as ecological character displacement. However, there are reports of character convergence in areas of overlap. Here we present a case study of sympatric convergence and allopatric divergence in form between two species, *Barnea candida* (Linnaeus, 1758), and *Pholas orientalis* Gmelin, 1791.They are members of closely related genera of marine bivalves from the eastern coast of India. So far as we know, this is the first report of a biological interaction between marine living bivalves. These two species are mud-boring deposit feeders. All of the characters here analyzed to show character shifting have adaptive significance or are proxy data for trophic apparatus. The present study involves more than 1,200 specimens collected from three sympatric and three allopatric sites that range over 1,000 km. Statistical analyses reveal that *B.candida* diverges more from its allopatric position than *P. orientalis* in sympatry. Character displacement generally takes place in sympatry to avoid or minimize competition for similar resources. The present two species have similar reproductive and feeding strategies. They also have similar ecological requirements for food and habitat. Because of these ecological and functional constraints, *Barnea candida* and *Pholas orientalis* could not reduce competition by resource partitioning. Instead, they compete and hence converge.

Key words: boring bivalves, Indian coast, sympatric convergence, ecological constraints.

INTRODUCTION

Species living in any area are always subjected to natural selection pressure, which has both physicochemical and biological attributes. Even when physicochemical parameters remain more or less constant, two related species mutually react in sympatry. This is known as biological interaction between two related species (Schindel & Gould, 1977). In a majority of cases, as studied in terrestrial animals or plants, the common reaction is what is known as ecological character displacement (Brown & Wilson, 1956). These authors stated, "When two species of animals overlap geographically, their differences are accentuated in the zone of sympatry and weakened or lost entirely in the parts of their ranges outside this zone" (p. 63). However, subsequent workers expanded this evolutionary process to include both divergence and convergence of relevant characters (Grant, 1972; Taper & Case, 1992). Divergent character displacement results from the fact that it allows competing species to reduce competition for resources, such as food and habitable space in sympatric areas. This is done by partitioning of the resources, which consequently leads to variation of characters related to foraging by the sympatric populations (Dunham et al., 1979; Schulter & McPhail, 1992; Robinson & Wilson, 1994; Scott & Foster, 2000).

This pattern of sympatric divergence and allopatric convergence has been observed in different organisms, including birds (Orians & Wilson, 1964; Grant, 1965, 1966), lizards (Schoener, 1970; Huey & Pianka, 1974), frogs (Blair, 1974), fishes (Schluter, 1994), tiger beetles (Pearson & Mury, 1979), plants (Grant (1994), and others (Dayan & Simberloff, 2005; and references therein).

However, biological interaction in sympatry can give rise to character convergence instead of displacement between two ecologically simi-

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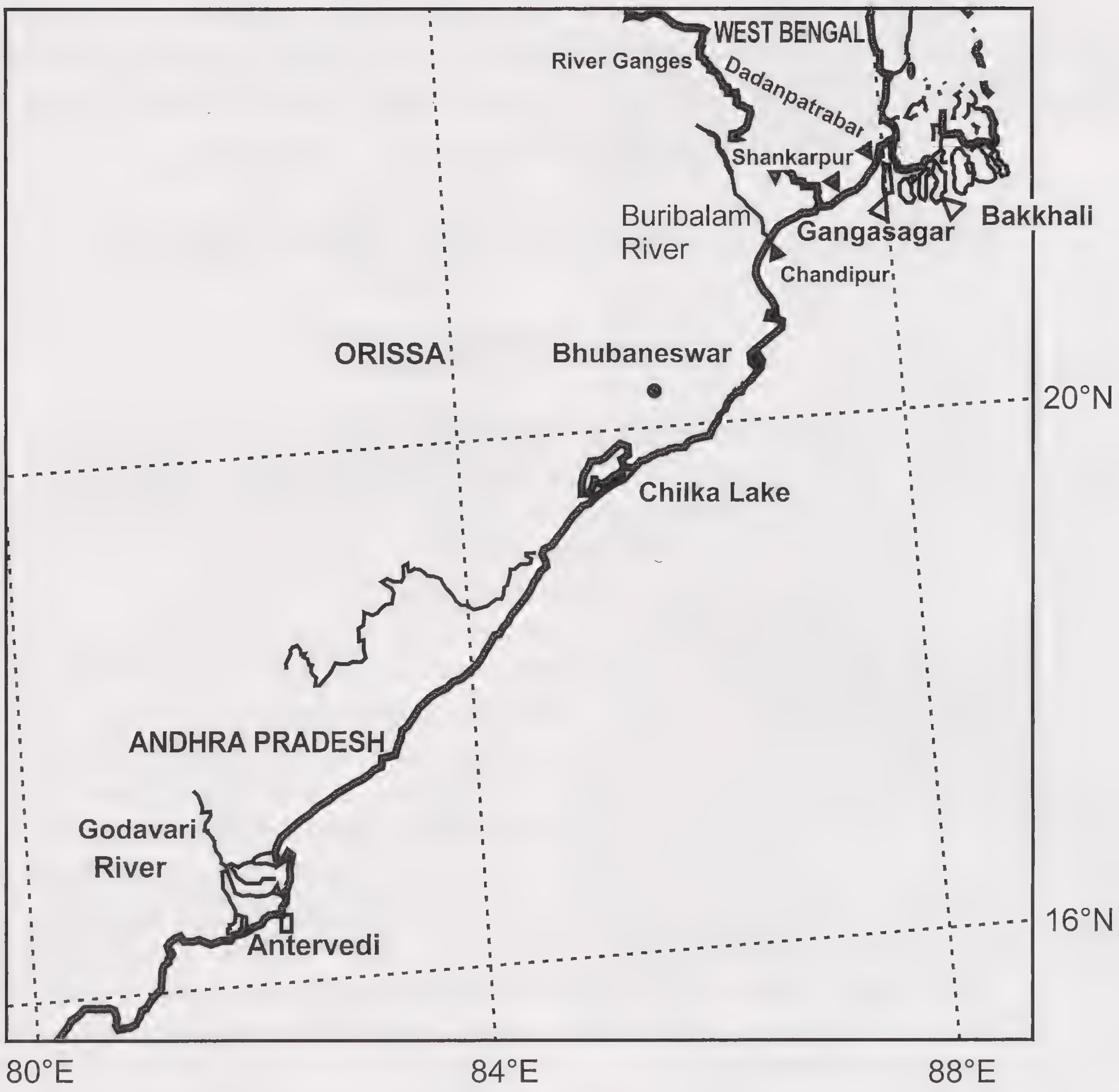

FIG. 1. Map of the coastline along eastern India, showing field sites of both sympatric (close triangle) and allopatric populations of *Barnea candida* (open triangle) and *Pholas orientalis* (rectangle).

lar species (Schoener, 1970; Grant, 1972, 1975; Wilson, 1975; Taper & Case, 1992). According to Cody (1973), character convergence can occur between closely related species or genera that can act in a very similar way if resources do not limit further population growth, and ecology or diet are very similar. He further stated that convergence is shown by characters of ecological significance. Convergence can also occur if sympatric populations try to monopolize habitats and convergent characters are related to territory establishment and maintenance (Cody, 1969, 1973; Grant, 1972; Arthur, 1982).

There are many theoretical models that explain both character displacement and convergence (Slatkin, 1980; Abrams, 1986, 1987; Taper & Case, 1992). But a general observation of these models is that under many circumstances at least the sizes of two competing taxa show parallelism or convergence rather than divergence (Dayan & Simberloff, 2005).

Molluscan examples of such biological interactions are now emerging. In a terrestrial gastropod community, two related species exhibit traditional allopatric convergence and sympatric divergence (Schindel & Gould, 1977). Subsequently, many workers studied land snails to demonstrate character displacement (Dayan & Simberloff, 2005), and some studies indicate that character displacement can be caused by environmental factors (Emberton, 1995). Marine examples of molluscan character displacement are available mainly from fossil taxa. Elredge (1968) studied character displacement in Pennsylvanian archeogastropod species. Sympatric character convergence has been studied by one of us (S. B.) along with others in Jurassic trigoniid bivalves (Rudra & Bardhan, 2006; Rudra et al., 2007), and eucycloceratiin ammonites (Jana et al., 2005). In the present endeavor, we have studied the interaction between two species of closely related genera of modern marine bivalves. This is the first known example of biological interaction between two established species of marine living Mollusca resulting in character convergence.

Barnea candida (Linnaeus, 1758) and *Pholas orientalis* Gmelin, 1791, are two boring Recent bivalves, belonging to the subfamily Pholadinae of the family Pholadidae (Turner, 1969). They are found in many areas of the world, but they have different distributional patterns on the eastern coast of India (Fig. 1). *Pholas orientalis* is restricted mainly to the southern part, whereas *B. candida* is found in the northeastern areas. The sympatric sites, where these two species live together, lie between. They are morphologically distinct and are well recognized in the literature (Turner, 1969). Here, we show that their morphological distinction becomes weak in sympatric populations, especially in size and other quantifiable morphological characters that have functional and ecological significance. Detailed statistical analyses involving univariate, bivariate, and multivariate methods support this observation.

Barnea candida and *Pholas orientalis* have similar ecological requirements for food and habitat. These constraints compel them to compete for the same resources, which perhaps results in the observed sympatric convergence.

MATERIALS AND METHODS

Several field trips were carried out in different seasons during 2005–2006. The studied area includes both allopatric and sympatric sites of *Barnea candida* and *Pholas orientalis*, ranging more than 1,000 km from their northeastern to southwestern limits. Allopatric sites of *Barnea candida* are Bakkhali and Gangasagar, which are located in the northeastern part of the studied area, whereas the only allopatric site of *P. orientalis,* Antarvedi, falls at the southern limit (Fig .1). About 1,200 samples were analysed. They were all dead and collected systematically. All of the samples were disarticulated in nature. We did not have any bias in collection. Where samples are sparse (e.g., Bakkhali and Chandipur), we collected all the samples encountered, including juveniles, sub-adults and adults. Where samples were plentiful (e.g., Gangasagar, Dadanpatrabar, Shankarpur, and Antervedi), we used a sampling design where we made randomly chosen 2 m x 2 m grids and collected all samples. We also collected valves that lay outside the grids, if they were of larger individuals, because one of our aims was to determine the change in body size in sympatry.

Preservation of the samples was very good. All of the delicate structural elements, for example, small anterior spines, ridges, apophyses, and extremely thin shells of both of the species, were still present in most specimens. These two species live within bottom-level community and do not have external ligaments, and therefore valves readily become disarticulated during exhumation after death. The presence of delicate structures suggests that the shells did not suffer much posthumous transport.

We do not claim that dead single valves we collected represent similar number of individuals. Theoretically, number of individuals should be half of the total number of single valves. But, as shells suffered postmortem transportation, ratios between left and right valves are not uniform in the collections. Left valves are generally more for both *Barnea candida* (ranges between 53.9% and 63.9%) and *Pholas orientalis* (52.3% to as high as 95.5% in allopetric Antervedi). Thus, it may be said that although studied 1,200 samples are completely disarticulated, they represent more than 600 individuals. Number of samples is thus quite sizable to quantify body size structures of living populations of two species.

The total number of hours spent searching for samples at a particular locality varied depending on local abundance of large adults and tide time. Total hours spent in searching samples were 12, 18, 14, 12, 48 and 12 for Bakkhali, Gangasagar, Dadanpatrabar, Shankarpur, Chandipur, and Antervedi, respectively.

We measured only the dead specimens, and the specimens can be time-averaged. Delicate shell characters cannot sustain long exposure to waves and currents for long. Even so, time-averaged death assemblages of marine molluscs could be more faithful representatives of the regional populations than living individuals (Kidwell & Flessa, 1995; Kidwell, 2001; Roy et al., 2003).

Barnea candida and *Pholas orientalis* are found only in muddy substrata of varying density. Muddy substrata are highly localized and are found only near estuary mouths. Because of their peculiar life mode, these two species have many functional morphological characters in common. Boring bivalves in general and pholadines in particular have a streamlined shape, extremely thin shells that are strongly inequilateral, gaping at both anterior and posterior ends, deep pallial sinuses, and small anteroventral spines for scraping within the stiff medium. These characters have functional significance, and some of them represent trophic

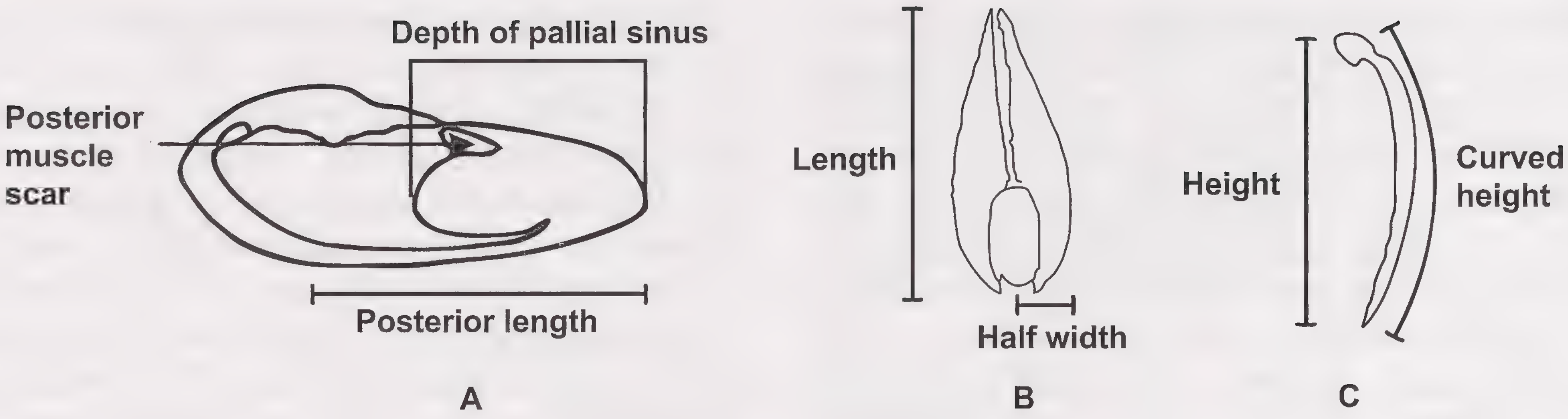

FIG. 2. General dimensions of pholadine bivalves measured in this study. A: lateral internal view; B: dorsal view; C: anterior transverse view.

structure. Pholadines are sessile benthic and deposit feeders (Stanley, 1970). They have long siphons used as trophic appendages to forage plant particulates from the sediment. The depth of the pallial sinus was used here as a proxy for the length of the siphons.

We have chosen seven characters to compare variation among allopatric and sympatric populations. These characters, we believe, have ecological and functional value, and *Barnea candida* and *Pholas orientalis* show interspecific diversity with respect to these characters. In addition, each also has its own genus-diagnostic, non-quantifiable stable characters, which make the two species taxonomically quite distinct. For example, *P. orientalis* has a pronounced smooth posterior end, a septate umbonal region, and spatulate apophyses, whereas *B. candida* has a simple umbo, rod-like apophyses, and entire ornamentation (Turner, 1969). These two species show convergence only in functional morphological characters, whereas the stable characters remain almost unchanged. The seven functional characters (Fig. 2) are:

1. Shell length – distance measured between the anterior and posterior tips.
2. Shell height – distance measured along the vertical line between umbo and ventral commissure.
3. Shell width – maximum width measured along the same plane where height has been measured. The value has been doubled to obtain the shell width of the intact animal.
4. Posterior length – distance measured between mid-umbonal line and posterior tip.
5. Depth of pallial sinus – distance measured between posterior end of the shell and point of maximum indentation of the pallial sinus.
6. Curved height – distance measured between umbo and ventral commissure along the shell surface.
7. Area of posterior muscle scar – product of the length along the maximum elongation of the muscle scar and the height perpendicular to it.

In addition, we have computed two shape indices, which are:

8. Relative inflation of the shell (shell width/shell height)
9. Relative convexity of the valve (shell height/curved height).

Because convergence in shape is not very much evident in multivariate analysis, as loadings of these shape data become masked by the influence of size data in first two discriminant functions, these characters are used only in univariate analyses.

From the univariate analyses, it is clear that the degree of convergence in all of the sympatric areas is not constant. To understand the nature of variation among sympatric areas, we also performed statistical analysis to determine the between-group Mahalanobis distances between each sympatric site with respect to allopatric sites.

Between-group variation between the two ecologically closely related species *Barnea candida* and *Pholas orientalis* has been studied in the allopatric and sympatric sites to understand and quantify the amount of morphological shifting. We have employed univariate (simple data display), bivariate (growth curves), and multivariate (Mahalanobis distance and discriminant function analysis) techniques to analyze the variation. Discriminant function analysis (DFA) involves group mean vectors, which are multivariate analogs of the means. Here DFA has been used to discriminate between *a priori* groups in terms of their group centroids to evaluate the patterns in morphological differences. The univariate data display the contribution of the individual variables to the multivariate distances.

TABLE 1. Mahalanobis distances among allopatric and sympatric populations of *Barnea candida and Pholas orientalis.* F values are shown in parentheses and are significant in 99% confidence level except in *P. orientalis* allopatric and sympatric populations.

	Barnea candida		*Pholas orientalis*	
	Sympatric	Allopatric	Sympatric	Allopatric
Barnea candida				
Sympatric	–	3.67210	9.78198	–
Allopatric	(8.74949)	–	–	19.81113
Pholas orientalis				
Sympatric	(29.33528)	–	–	1.25287
Allopatric	–	(25.49808)	(1.81384)	–

Most bivalves do not generally have any recognizable adult characters and growth is continuous. However, growth lines become crowded, and constrictions appear near the ventral margins in the present species during adult stages. We have measured 15 large specimens from each locality for our analyses.

We have done all of the measurements, except the curved height, with digital slide calipers with a precision of 0.01 mm. Statistical analyses were performed with SPSS ver. 15.0 statistical software packages (SPSS ver. 15.0, licensed and registered to Palaeontology Division, Geological Survey of India, Kolkata).

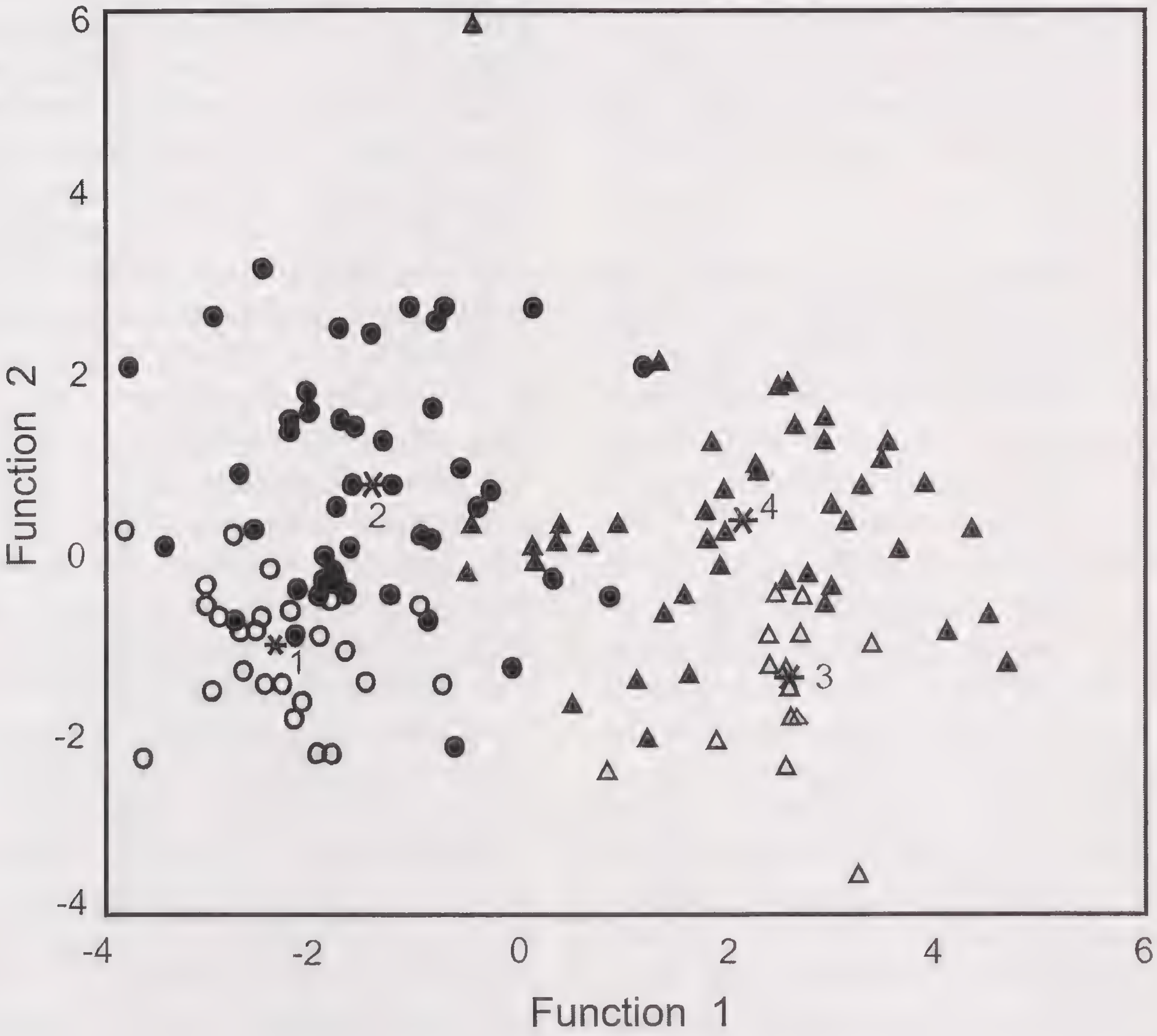

FIG. 3. Plot of all specimens on the first two functions of a four-group canonical discriminant function analysis. Circles represent *Barnea candida* and triangles represent *Pholas orientalis.* Open symbols represent allopatric populations and closed symbols represent sympatric populations.

TABLE 2. Loading of seven morphological characters onto the three discriminant functions of a four-group solution.

	Function 1	Function 2	Function 3
Shell length	0.636	1.742	-0.194
Shell height	-0.152	0.026	-0.782
Shell width	-0.228	0.465	-0.731
Posterior length	1.126	-1.989	0.409
Depth of pallial sinus	-0.052	0.369	-0.680
Curved height	-0.754	-0.699	1.480
Size of posterior muscle scar	-0.100	0.715	0.565
Total fraction of information explained	84.7%	14.1%	1.2%
Eigen value	4.31	0.82	0.08

RESULTS

The matrix generated by the Mahalanobis distances shows the relationships of the allopatric and sympatric populations of the species in terms of the relative distances between group centroids in reduced space. When we performed such an analysis on the four groups (sympatric and allopatric *Barnea candida* and *Pholas orientalis* populations), we found that these two species are more alike in sympatry than allopatry. The Mahalanobis distance and its F values of significance of the *Barnea candida* and *Pholas orientalis* populations are shown in Table 1. The F values indicate that, except in allopatric and sympatric populations of *P. orientalis*, the Mahalanobis distances are significant in 99% confidence level between all other populations. Mahalanobis D^2 based on all of the characters for allopatric populations of both of the species is 19.81. On the other hand, the Mahalanobis D^2 between the species in sympatry is 9.78. This significant reduction in Mahalanobis distance in sympatry than that of allopatry strongly suggests biological interaction leading to character convergence. Functions 1 and 2 of Figure 3 explain 84.7% and 14.1%, respectively, of all of the information. Table 2 shows loading of the original seven measures onto the three discriminant functions, along with their associated eigen values. It reveals that allopatric populations are separated mainly in shell length and curved height manifested by a wide separation along Function 1. The Mahalanobis distance between centroids of allopatric and sympatric *B. candida* is 3.67 (F value 8.75, significant at 99% confidence level), whereas that of *P. orientalis* is 1.25. This indicates that *B.candida* shifts more in sympatry than *P.orientalis*, and, as such, it is more dominant.

The contribution of individual measures onto the multivariate difference can also be expressed as univariate graphs. Table 3 shows the mean and standard deviations of all of the measures across all of the locations. Univariate plotting of individual characters also supports the result derived from multivariate analysis. Plotting of length data (Fig. 4) across all of the locations reveals that both species have shifted their character value in the presence of the other and have maintained nearly a fixed difference across all of the sympatric sites. It becomes apparent that in three sympatric areas (i.e., Dadanpatrabar, Shankarpur, and Chandipur), where they experience competition, their size comes reasonably close to Hutchinson's ratio (1:1.27 at Dadanpatrabar, 1:1.30 at Shankarpur, and 1:1.33 at Chandipur). This ratio indicates that these species maintain "an effective strategy for avoiding competitive elimination" (Clarkson, 1998: 49). Interestingly, the difference between the ratios is most pronounced between the allopatric zones (1:1.51), where they live separately. Figure 4, depicting the variation in depth of the pallial sinus, shows a parallel trend with that of length data. Their ratios in sympatry are again close to Hutchinson's values (1:1.25 at Dadanpatrabar, 1:1.31 at Shankarpur, 1:1.29 at Chandipur). Posterior length (Fig. 4) also shows a broad parallelism in sympatry. In Figure 4, which depicts variation in the size of the posterior muscle scar, the two species populations show strong convergence and the sympatric lines intersect.

The inflation graph (Fig. 4) shows strong convergence, and the shell convexity graph (Fig. 4) shows most of the sympatric values lying

TABLE 3. Means and standard deviations of populations of *Barnea candida* and *Pholas orientalis* at all sampled locations. L = Shell length; H = Shell height; W = Shell width; PL = Posterior Length; PS = Depth of pallial sinus; CH = Curved height and MS = Size of posterior muscle scar.

Population	L	H	W	PL	PS	CH	MS
Allopatric *Barnea candida*	61.7185 ± 5.26076	24.7922 ± 6.32157	22.9163 ± 2.39811	44.1415 ± 4.71454	32.9704 ± 5.17701	33.6667 ± 3.50823	27.9978 ± 7.20817
Allopatric *Pholas orientalis*	98.7486 ± 4.26422	28.5979 ± 1.40958	27.9100 ± 1.45028	78.0593 ± 2.70442	49.4693 ± 13.07397	40.7857 ± 2.19014	52.3293 ± 14.67749
Sympatric 1 *B.candia* Dadanpatrabar	79.1308 ± 6.39262	28.4775 ± 2.05778	27.1050 ± 2.86637	57.1700 ± 6.44202	42.3467 ± 3.95815	39.3333 ± 3.25669	58.4842 ± 6.39061
Sympatric 1 *P. orientalis* Dadanpatrabar	100.2327 ± 12.12219	30.4200 ± 2.79504	29.8947 ± 3.63078	76.3873 ± 10.23077	53.1173 ± 6.68241	41.0373 ± 4.74586	64.3527 ± 12.82142
Sympatric 2 *B.candida* Shankarpur	86.4700 ± 7.11863	30.4757 ± 2.87622	29.6186 ± 2.96508	56.9586 ± 5.49308	45.3086 ± 3.23614	42.9286 ± 5.29825	66.4950 ± 10.10476
Sympatric 2 *P. orientalis* Shankarpur	112.6293 ± 5.28341	33.4836 ± 1.93044	33.0386 ± 3.05858	84.3771 ± 6.10256	59.2114 ± 3.97846	46.2143 ± 3.42342	65.0593 ± 16.77995
Sympatric 3 *B.candida* Chandipur	65.2307 ± 4.25574	23.5233 ± 1.69910	23.2760 ± 1.70215	46.6333 ± 3.75431	35.9840 ± 2.79390	32.2667 ± 4.23365	37.0593 ± 12.48410
Sympatric 3 *P. orientalis* Chandipur	86.8960 ± 16.19034	26.6610 ± 4.17750	25.8500 ± 4.48648	65.8450 ± 13.90320	46.5680 ± 9.58034	35.1000 ± 6.52261	61.1040 ± 20.41541

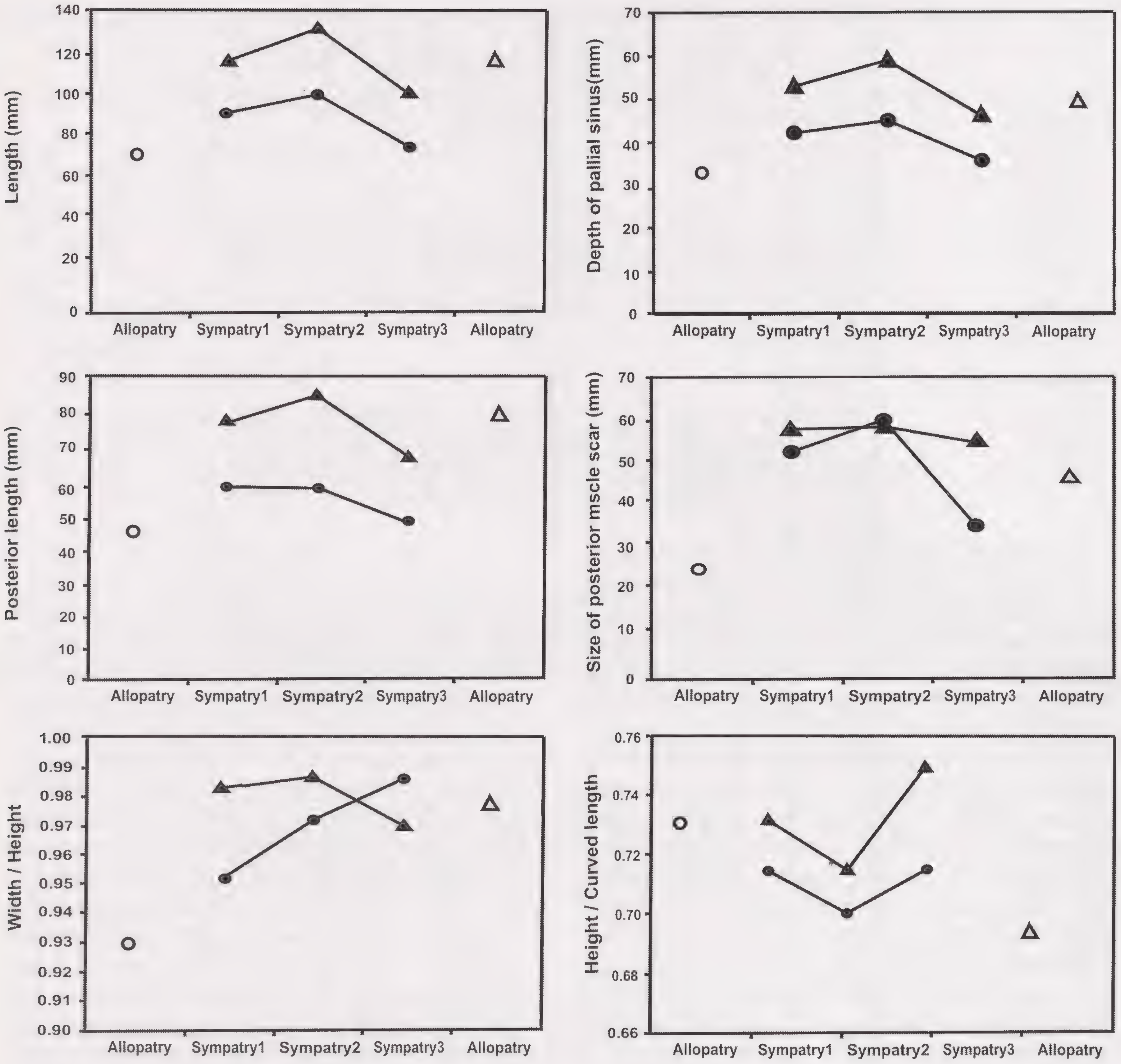

FIG. 4. Univariate plots of different shell morphologies in allopatric (open symbols) and sympatric (closed symbols) populations of *Barnea candida* (circles) and *Pholas orientalis* (triangles). Solid lines connect means of sympatric samples of each species.

intermediate between two extreme allopatric values, especially for *Barnea candida.* In different allopatric areas, this species shows high intraspecific genetic variability with respect to shell width and valve curvature. At Gangasagar, the average shell length of *B. candida* (mean 59.11 mm) is smaller than that at Bakkhali (mean 63.86 mm), but width/height and height/curved height ratios are always greater. This suggests that these two characters are independent of size. However, in sympatry with competing *Pholas orientalis*, the values of these characters always show increasing trends indicating clearly the role of biotic interaction. *Pholas orientalis* shows a somewhat mixed reaction with respect to these two characters. In some sympatric areas, shell inflation remains more or less the same and in others, it slightly decreases or increases. However, it strongly reacts with respect to the shell curvature data. In all sympatric areas, *P. orientalis* diverges from its allopatric values and shows a high degree of shell streamlining. Overall, the shape data tend to show broad parallelism among the sympatric areas like those of the size-induced characters discussed above.

Mahalanobis distances between sympatric populations of *Barnea candida* and *Pholas*

TABLE 4. Distribution of specimens of *Barnea candida* and *Pholas orientalis* collected at all sampled localities.

	Bakkhali	Gangasagar	Dadanpatrabar	Shankarpur	Chandipur	Antervedi
Barnea candida	95	350	300	500	75	–
Pholas orientalis	–	–	75	155	15	350

orientalis were 12.43, 19.82, and 12.84, respectively, for Dadanpatrabar, Shankarpur, and Chandipur. Interestingly, Mahalanobis D^2 between each allopatric *B. candida* and *P. orientalis* population consistently had greater values. For example, D^2 between Bakkhali *B. candida* and Antervedi *P. orientalis* is 24.51 and Gangasagar *B. candida* and Antervedi *P. orientalis* is 29.23. At Shankarpur, shifting of *B. candida* from its allopatric populations (D^2 11.67 and 13.60, respectively, for Bakkhali and Gangasagar) is greater than that of *P. orientalis* (D^2 6.45 for Antervedi). On the other hand, at Chandipur, *B. candida* underwent less of a shift (D^2 1.68 and 1.38, respectively, for Bakkhali and Gangasagar) than did *P. orientalis* (D^2 4.01). At Dadanpatrabar, the Mahalanobis distances between allopatric and sympatric *B. candida* were 8.34 and 9.91, respectively, for Bakkhali and Gangasagar; the difference between allopatric *P. orientalis* and sympatric *P. orientalis* was 2.05. This pattern of asymmetrical convergence and varied degree of shifting of character values from allopatric populations shown by both species indicates that the degree of convergence is not only a function of the presence or absence of competition, but also is a function of the intensity of competition. The intensity of competition is manifested by the relative abundance of each species at each location (Table 4).

With the help of another observation of discriminant function analyses (Table 5), we can further confirm the finding that sympatric populations of both species come closer than in allopatry. We found that identification phase of this analysis correctly assigned the allopatric populations to their respective taxonomic groups. The re-identification was done in the discriminant analysis. In the DFA, the degree of success of classification is measured by calculating the error or misidentification rate (Shindel & Gould, 1977). The previously made groups, using qualitative characters of taxonomic identification and presence-absence data (i.e., the two species and allopatric and sympatric populations) were tested and re-allocated using the discriminant function calculated from the seven variables. It is seen that the error rate while discriminating between allopatric populations of *Barnea candida* and *Pholas orientalis* is always 0, whereas in the sympatric populations there is an overlap and the error rate increases to 6.7% and 11.1% respectively. This degree of overlapping can be attributed to morphological convergence in sympatry.

Because allopatric and sympatric populations of each species show morphological variation, we wanted to determine the variation of population structure of each species between allopatry and sympatry. We collected samples ranging from very small to very large size, and we

TABLE 5. Result of discriminant function analyses identification phase: hits and misses from rows (actual group membership) into columns (assigned membership).

	Allopatric		Sympatric	
	Barnea candida	*Pholas orientalis*	*Barnea candida*	*Pholas orientalis*
Allopatric				
Barnea candida	100	0	0	0
Pholas orientalis	0	86.7	0	13.3
Sympatric				
Barnea candida	22.2	0	71.1	6.7
Pholas orientalis	0	20.0	11.1	68.9

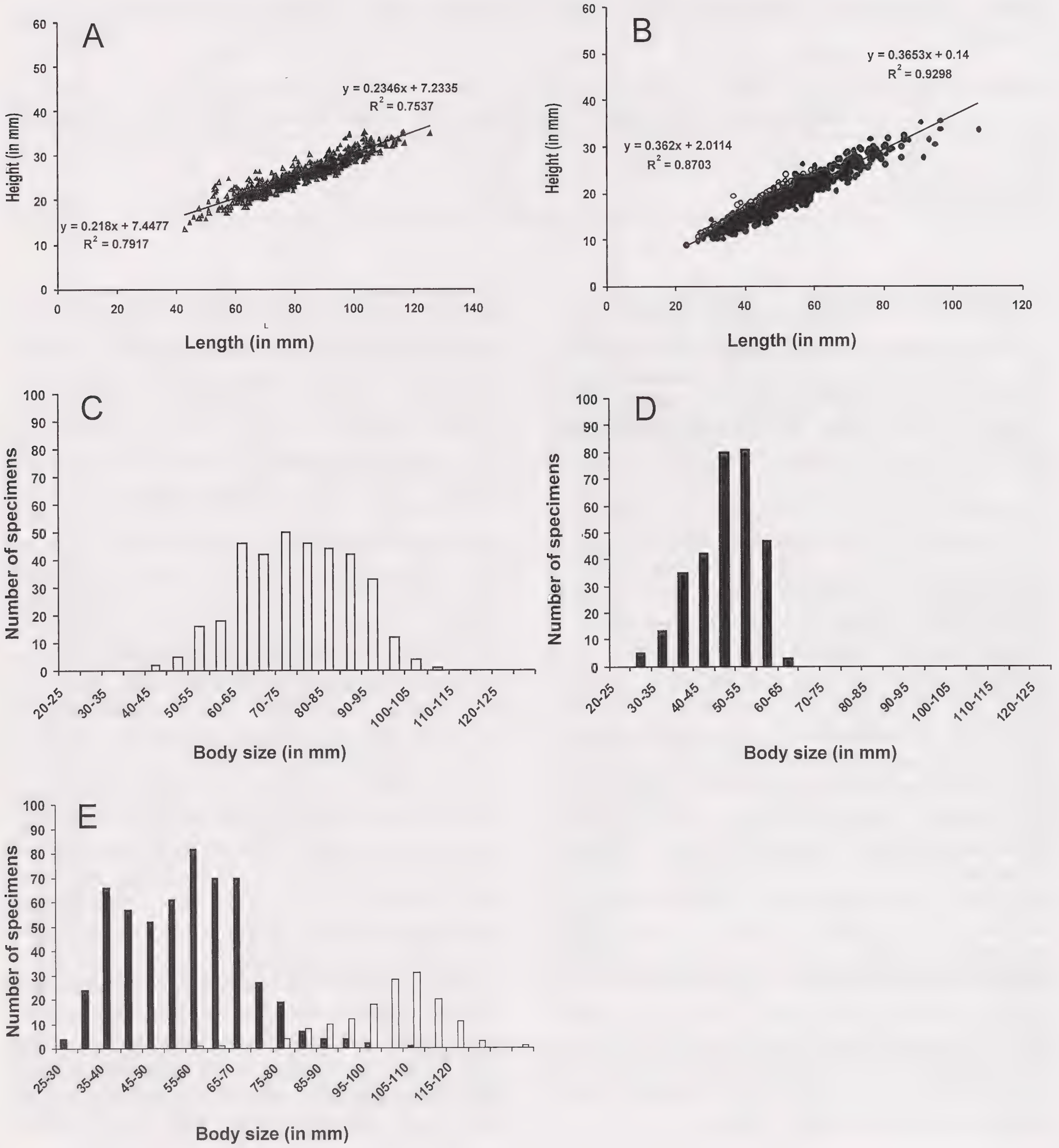

FIG. 5. Growth curves for allopatric (open symbols) and sympatric (closed symbols) populations for *Barnea candida* (A) and *Pholas orientalis* (B). Histograms of body size of specimens of both species in the same allopatric populations (Gangasagar for *B. candida* and Antervedi for *P. orientalis*) are shown in C, D, and E, respectively. Solid bars represent *B. candida* assemblages, whereas open bars represent *P. orientalis*. Locality details in Fig. 1.

performed bivariate analyses involving height and length (Fig. 5A, B), as well as histograms (Fig. 5C–E), to show distribution of each normal assemblage in different areas.

All of these analyses revealed that *Barnea candida* and *Pholas orientalis* have become morphologically closer to each other in sympatry, and they have diverged in allopatry. This convergence in sympatry is evident in both size and shape data.

DISCUSSION

In the south, where *Pholas orientalis* lives alone, the eastern Indian coast is a more exposed shore, whereas allopatric populations of *Barnea candida* are from sheltered areas belonging to the Sunderban delta. Therefore, it can be argued that the changes in body size and other shell characters could be due to nonbiological factors. There are examples where coastal populations show clinal variation due to differing degrees of water turbulence (Newkirk & Doyle, 1975; Peel, 1987) or temperature (Yom-Tov, 2001) and food. Clinal variation is seen mostly in epifaunal organisms. Mud-boring infauna, such as the present species, are protected within their domicile from water turbulence and can remain indifferent. Many other infaunal bivalves, living in either sympatric or allopatric areas of *B. candida* and *P. orientalis*, do not show size or other variation (Rao et al., 1991, 1992; Mahapatra, 2001). This suggests that there is little or no difference in the abundance or quality of food among areas (direct measurement of availability of food particles was beyond the scope of this study). Other than competition, the benthic community faces predation pressure, and therefore the degree of predation might also exert selection pressure resulting in morphological variation. The eastern coast of India has a high diversity of predatory marine animals, such as fishes, crabs, and gastropods (Rao et al., 1991, 1992). Bivalves are mostly preyed upon by carnivorous muricid and naticid gastropods. Many dead bivalve shells littering Indian coastal areas bear characteristic boreholes made by such naticid gastropods as *Natica* and *Polinices*, or commissural breakage (personal observation). These kinds of predatory marks have also been observed in other areas (Carriker & Yochelson, 1968; Vermeij, 1987). Among the more than 1,200 specimens we studied, not a single specimen shows evidence of boring (members of two genera of naticids have prey-specific diets and target mostly shallow burrowing bivalves living in sandy habitats). Although gaping bivalves are often attacked through the gape (Vermeij, 1980; Ansell & Morton, 1987), this is not the case here. *Barnea candida* and *P. orientalis* always live in a firm muddy habitat and remain in situ within deep protective borings and are not subject to substantial predation pressure from gastropods. It, therefore, appears that size and other morphological variation shown by the present two species in sympatry from their respective allopatric areas are mainly due to their own biological interaction.

Such size indices as shell length, depth of the pallial sinus, and posterior length, show maximum variation in sympatry. In the case of *Barnea candida,* shell length increases significantly from allopatric sites (Bakkhali and Gangasagar) to sympatric sites (Dadanpatrabar and Shankarpur, $p < 0.001$). Interestingly, shell length dose not vary significantly within these sympatric sites ($p > 0.001$). In the case of *Pholas orientalis*, shell length increases significantly at one sympatric area, that is, Shankarpur ($p < 0.001$); however, this reduction is not statistically significant ($p > 0.001$) at Chandipur. Univariate analysis shows broad parallelism in the distribution of shell length values among the sympatric populations of the two species (Fig. 4). However, at all sites, their shell length ratio remained close to Hutchinson's value. Pholadines are strongly inequilateral (posterior part much greater than anterior part); therefore, further elongation of the shell at sympatric sites, for example, at Shankarpur, also was manifested in an increase in posterior length (for *B. candida*, $p < 0.001$; difference is marginally insignificant for *P. orientalis,* $p > 0.001$). In asymmetrical boring bivalves, the posterior part is generally larger because it accommodates long siphons. The distribution of the depth of the pallial sinus also shows this broad parallelism with shell length in sympatry (Fig. 4). In the case of *B. candida*, the allopatric sites are not significantly different ($p > 0.001$) from one another. However, statistically significant difference ($p < 0.001$) exists between allopatric and sympatric sites. On the other hand, *P. orientalis* shows less variation in sympatry ($p > 0.001$). This perhaps, as mentioned earlier, is due to the fact that *P. orientalis* is less dynamic. In boring bivalves, especially in pholadines, there occurs a unique character shift, that is, the anterior adductor muscle scar migrates on the umbonal region. To minimize resistance against abrasion within the stiff substratum, they thicken their umbonal regions by refolding the shell, and in this process, they have lost their external ligament. Shifting the anterior adductor muscle on the umbonal region now helps the pholadine to open the valves, and it is functionally analogous to the didactor muscle in brachiopods (Pojeta, 1987). The posterior adductor muscle is now used alone to close the valves. Repeated opening and closing of valves occurs when the bivalves burrow within the substratum (Stanley, 1970). Therefore, a more powerful muscle is required to burrow more deeply. Sympatrically, both species have increased their muscle scar sizes, and we have already shown that they have also increased their shell length and depth of pallial sinus to access deeper substrata. At Shankarpur, where competition is most intense, they have almost identical muscle scar sizes.

From all of the statistical analyses, it is clear that *Barnea candida* and *Pholas orientalis* show morphological convergence in sympatry and divergence in allopatry. In most of the other cases, competition leads to divergent character displacement. This is because species reduce competition by partitioning such resources as food or space. Resource partitioning results in modification of characters of ecological significance. However, in the present study, this is not the case. Bivalves as a class have a specific diet. Being deposit or suspension feeders, bivalves could not reduce competition by food resource partitioning. The only other resource that the competiting species could reduce overlapping is the habitat. However, habitat segregation of the present two species is not possible for two reasons. First, they are mud borers and therefore cannot migrate to nearby sandy habitat. Secondly, the mud flats are spatially limited, found mainly near the estuary mouths. Because of these similar ecological requirements, the two species accommodate competition rather than avoid it. Because they are borers, they try to segregate the habitat vertically. However, in allopatric Bakkhali and sympatric Chandipur, *Barnea candida* lives at depths of 15 to 20 cm below the surface (Rao et al., 1991, 1992). There is no published data on depths of *P. orientalis* on the eastern coast of India, but elsewhere they are reported to bore to depths of at least 40% more than their shell lengths (Pinn et al., 2005). It appears that the two species have more or less the same depth limit within the substratum and therefore, when subjected to competition, they try to segregate by depth. This is evidenced by significant different depths of the pallial sinus.

It is already shown that the degree of convergence varies from place to place in the overlapping areas. At Dadanpatrabar and Chandipur, these two species show maximum convergence (D^2 11.30 and 12.91, respectively). Although degree of convergence is similar for these two areas, the reaction of each of the species is not very similar. At Chandipur, *Pholas orientalis* decreases shell length (Fig. 4) from the value of its allopatric populations, whereas *Barnea candida* almost remains neutral. At Dadanpatrabar, *B. candida* reacts more strongly by increasing shell length, depth of the pallial sinus, and posterior length, whereas *P. orientalis* stays more or less neutral. Relative population size of each species differs in sympatric areas. Although *B. candida* is always numerically superior in every site, the relative abundance of *P. orientalis* varies (Table 4). Because *P. orientalis* is very sparse at Chandipur, it appears that *B. candida* does not experience competitive pressure and therefore remains unchanged. *Pholas orientalis*, on the other hand, has significantly reduced its shell size over that of its allopatric population. At Chandipur, both species face steep competition for habitat from other deep-boring bivalves, such as *Sanguinolaria acuminata* Deshayes (1857), *Glauconome sculpta* G. B. Sowerby III (1894), and *Laternula truncata* Lamarck (1818) (Rao et al., 1991,1992; personal observation). Many of these species live deeper within the substratum; *L. truncata* burrows more than 1 m (Rao et al., 1991, 1992). Therefore, space left for the competing *P. orientalis* coincides with that of *B. candida.* At Dadanpatrabar, the other boring species are absent and *P. orientalis* is more abundant than at Chandipur. In many marine taxa, when habitat is a limiting resource, organisms tend to monopolize it (Raup & Stanley, 1978). They are, therefore, locked in severe competition in occupying this habitat. The only direction that they can move for ecological segregation is down into the substratum. From Fig. 4, it is clear that both species have become larger, more elongated with larger siphons, and show almost parallel trends in sympatry. The intensity of competition is best evident at Shankarpur, where each species has a robust population size; they are largest here, with deepest pallial sinuses. However, *P. orientalis* always remains relatively larger than *B. candida* and this way maintains better accessibility within sediments.

Barnea candida and *Pholas orientalis* have wide biogeographic distributions (Turner, 1969) and interestingly, an internet search (www.specimenshells.net, www.conchology.be, www.glaucus.org.uk, www.shell.kwansei.ac.jp) reveals that outside India they have allopatric distributions. We cannot assume that these website data are unbiased representations of body size distributions and are statistically robust. However, there is an anthropogenic bias that collectors often favour larger specimens (Roy et al., 2003), and these data may provide a baseline against which present body size distributions can be compared. Below we

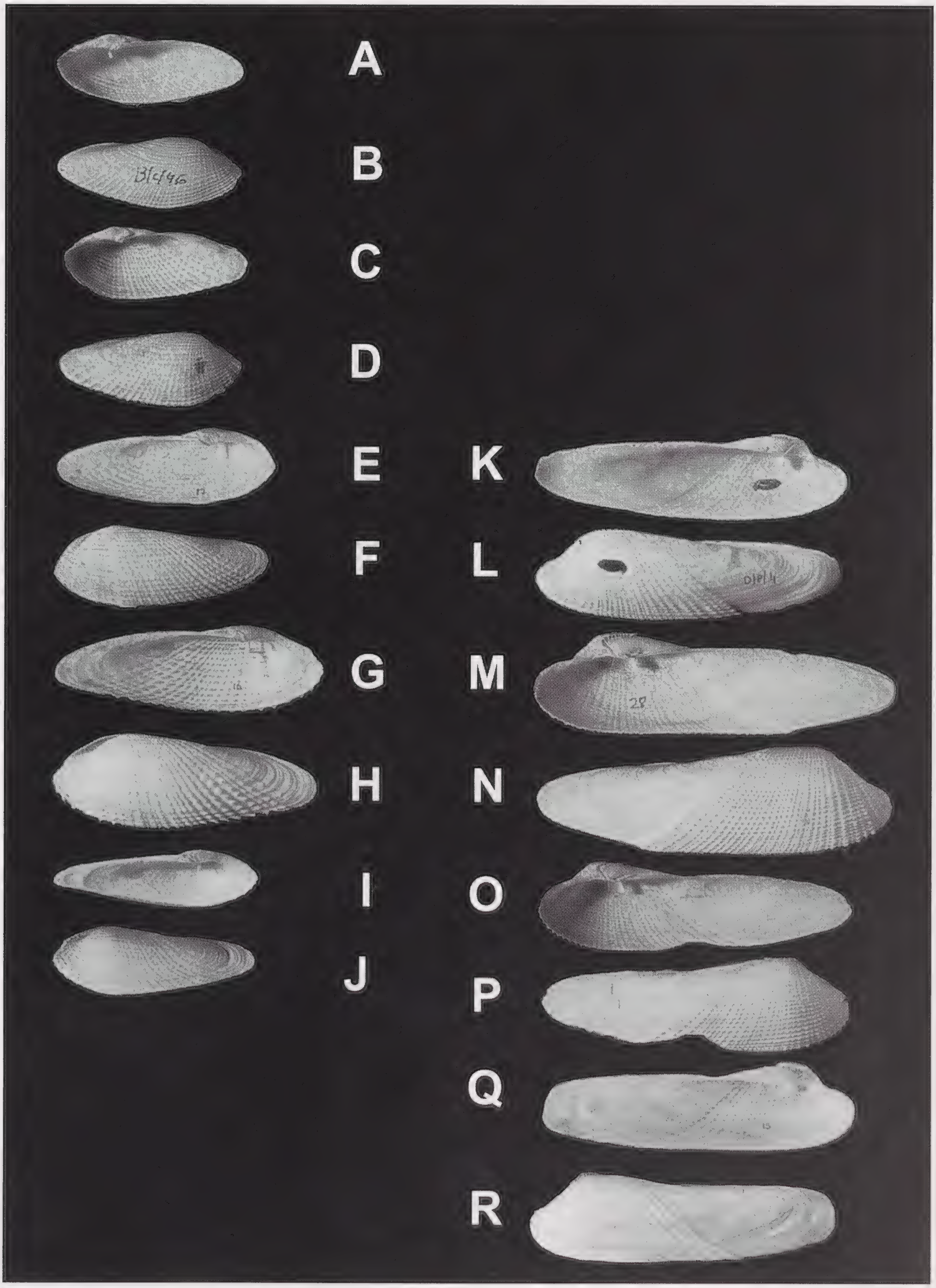

FIG. 6. Sympatric and allopatric populations of *Barnea candida* (left) and *Pholas orientalis* (right). Note that the shells of the two species are more similar in sympatry (compare E–J and K–P) than in allopatry (A–D and Q–R). The localities (also see Fig. 1) are: Bakkhali (A–B), Gangasagar (C–D), Dadanpatrabar (E–F and K-L), Shankarpur (G–H and M–N), Chandipur (I–J and O–P), and Antervedi (Q–R).

mention size ranges (if samples are few) and averages (if they are more than 10) provided by the websites. *Barnea candida* is mostly found in different areas in Europe, and their average size distribution is as follows. In France, body length ranges between 3.6 and 5.9 cm (www.conchology.be), whereas it ranges between 2.5 and 3.9 cm in England (www.glaucus.org.uk); maximum length in Spain is about 3 cm (www.shell.kwansei.ac.jp). Shell sizes of most of the European allopatric populations are smaller than the Indian allopatric populations, and in sympatric Shankarpur, India, the average length (8.6 cm) perhaps, is the greatest length recorded for *Barnea candida* in the world. Similar size ranges of *Pholas orientalis* in other areas also support this observation. Allopatric populations in Thailand (www.conchology.be) have average lengths of 9.22 cm, and in Singapore maximum length is 8.75 cm (Turner, 1969). These data are close to the size data of Indian allopatric distribution (9.87 cm at Antervedi). But, in the Philippines, the maximum size ranges from 11.2 to 13.4, cm with an average value of 12.2 cm (www.specimenshells.net; www.shell.kwansei.ac.jp). This is, however, slightly larger than the Indian sympatric value of 11.26 cm at Shankarpur.

All of the analyses reveal that both species show morphological variation between sympatric and allopatric populations. Bivariate growth curves (Fig. 5A), involving shell length and shell height, were performed to evaluate patterns of variation from early ontogeny. They demonstrate that the sympatric *Barnea candida* population has a smaller shell height value than that of the allopatric population, suggesting that competition has led this species to become more streamlined to penetrate deeper within substratum from early ontogeny. The bivariate graph of shell length and shell height also supports sympatric convergence, because growth trajectories come closer in sympatry than in allopatry.

Thus far, we have seen that the two species show morphological convergence in sympatry due to competition resulting from very similar ecological requirements (Fig. 6). However, other than food and habitable space, species try to avoid intraspecific hybridization (Schindel & Gould, 1977). At Gangasagar and Antervedi, the allopatric sites of *Barnea candida* and *Pholas orientalis*, respectively, and at Shankarpur, the main sympatric site, we have made fresh collections during June–August 2006. The histograms (Fig. 5C–E) of these populations reveal that the breeding seasons of the two species must be different. We have found smaller sizes of *B. candida* in allopatry and sympatry, suggesting that these monsoon months are the breeding season of *B. candida*. But we have not found any smaller samples of *P. orientalis* in sympatry, which are present in allopatry. It could be that there is a delay in the onset of the breeding season of *P. orientalis* in sympatry.

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PALAEOHETERODONT PHYLOGENY, CHARACTER EVOLUTION, DIVERSITY AND PHYLOGENETIC CLASSIFICATION: A REFLECTION ON METHODS OF ANALYSIS

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ABSTRACT

Graf and Cummings (2006), hereafter referred to simply as "G & C", provided phylogenetic analyses of a three-partition data set in order to (1) examine the higher level evolutionary relationships within the Palaeoheterodonta, (2) estimate the history of character state change, and (3) develop a phylogenetic classification for the group. However, portions of the available *COI* DNA sequence data, for multiple terminals, were omitted from G & C's phylogenetic analyses, and no attempt was made to explicitly account for the documented saturation in the *COI* data partition. In order to evaluate the effects of these omissions, we performed Bayesian inference (BI) as well as maximum parsimony (MP) analyses on G & C's combined evidence (CE) matrix that included all of the ingroup *COI* sequences contained in G & C, plus the omitted outgroup *COI* sequences. We conclude that G & C's *COI* DNA sequence omissions, when combined with MP analyses not accounting for *COI* saturation, negatively affected the topologies of the best trees obtained from phylogenetic analyses of the CE matrix. This conclusion questions the utility of G & C's inferences regarding palaeoheterodont bivalve character evolution as well as the taxonomic classification drawn from its preferred topology. For example, counter to G & C's inferences, our BI and "transformed *COI*" MP analyses determined that unionoid oyster conchology has evolved multiple times, and all of our phylogenetic analyses indicate that the Etheriidae (*sensu* G & C) is not monophyletic. However, it should be noted that, to date, no phylogenetic analysis of this data set has robustly estimated all basal nodes within the Unionoida. Therefore, any inferences regarding unionoid bivalve character evolution, diversity and classification drawn from these topologies should be considered weakly supported.

Key words: Palaeoheterodonta, Unionoida, Bayesian phylogenetics, maximum parsimony, phylogenetic classification, *COI*, *28S*, morphology.

INTRODUCTION

Graf & Cummings (2006), hereafter referred to simply as "G & C", provided phylogenetic analyses of a three-partition (partial *COI*, partial *28S* and morphology/life history characters), combined evidence (CE) data set in order to (1) examine the higher level evolutionary relationships within the Palaeoheterodonta, (2) estimate the history of character state change, and (3) develop a phylogenetic classification for the group. Given the unsettled nature of relationships within the constituent order Unionoida (= freshwater mussels; reviews in Roe & Hoeh, 2003; Walker et al., 2006; Figs. 1, 2, herein), we applaud this effort.

But, given G & C's acceptance of a combined evidence philosophy, we find it curious that G & C did not include the available, homologous *COI* sequences (actually F*COI* mitochondrial DNA [mtDNA] sequences; see Breton et al., 2007, for a recent review of doubly uniparental inheritance of mtDNA) for the two outgroup terminals, *Mytilus* and *Astarte*, in any of its phylogenetic analyses. Additionally, G & C did not include the available, homologous *COI* sequences for three ingroup terminals, *Coelatura*, *Pseudomulleria* and *Obliquaria* in the analysis that produced its preferred tree (G & C: fig. 3; from which the higher-taxonomic relationships are portrayed in G & C: fig. 4 and Fig. 1, herein). G & C's omission of these ingroup *COI* sequences resulted in

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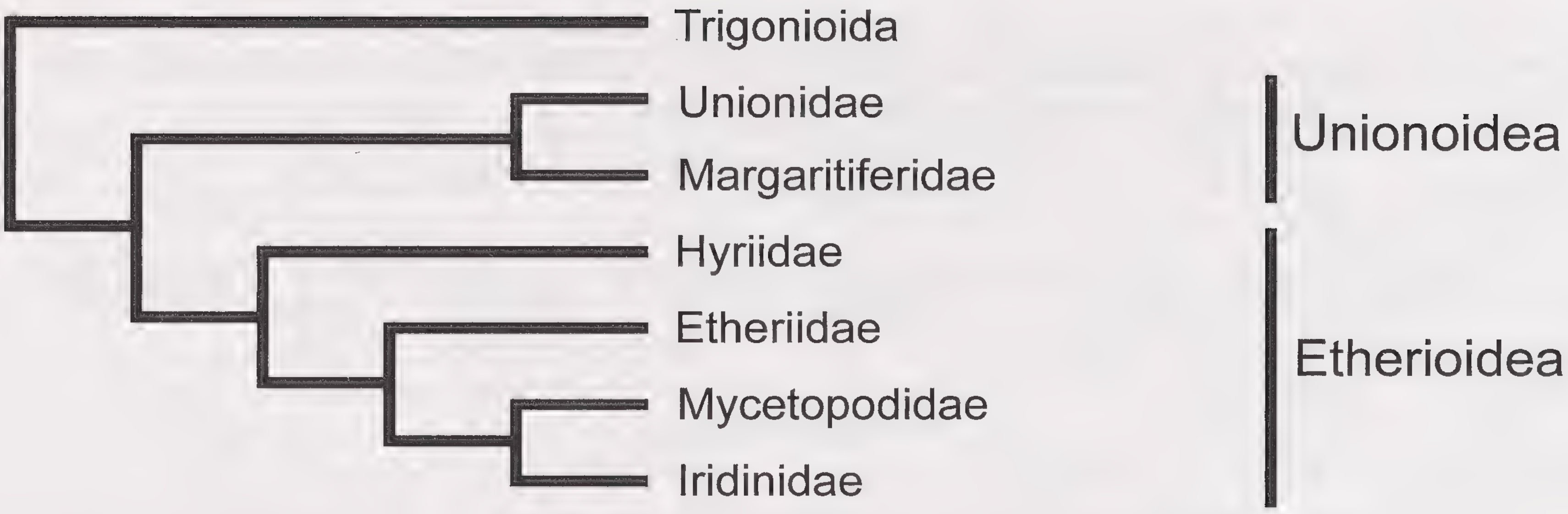

FIG. 1. Summary tree showing the higher level relationships within the order Unionoida as portrayed in G & C (2006: fig. 4). Familial and superfamilial nomenclature: from G & C (2006).

the removal of all of the available DNA characters for two of these three terminals.

We also found curious G & C's position regarding the proper method of analysis for *COI* DNA sequences. Despite previous publications' documentation of *COI* DNA sequence saturation (e.g., Hoeh et al., 1998; Graf & Ó Foighil, 2000) at this time depth (> 200 my; Watters, 2001), G & C's phylogenetic analyses did not encompass methods designed to compensate for the acknowledged saturation in the *COI* DNA sequences. G & C gave no explanation for this apparent incongruity.

Thus, in order to evaluate the effects of G & C's (1) outgroup and ingroup *COI* DNA sequence omissions and (2) analysis of *COI* DNA sequences without explicitly addressing the issue of saturation on inferences regarding palaeoheterodont bivalve evolutionary relationships, we performed Bayesian inference (BI) as well as maximum parsimony (MP) analyses on G & C's CE matrix that included all of the data contained in G & C plus *Mytilus* and *Astarte COI* DNA sequences. The MP analyses were performed on CE matrices containing both non-transformed and transformed (= 3rd codon position transitions deleted) *COI* sequences. Both the BI and "transformed *COI*" MP analyses represent attempts to compensate for the saturation contained within the *COI* DNA sequence data partition. We conclude that G & C's *COI* DNA sequence deletions, when combined with MP analyses without "corrections" for *COI* saturation, negatively affected the topologies of the best trees obtained from phylogenetic analyses of the CE matrix. This conclusion questions the utility of the inferences regarding palaeoheterodont bivalve character evolution as well as the taxonomic classification drawn from G & C's preferred topology (G & C: figs. 3, 4; Fig. 1 herein).

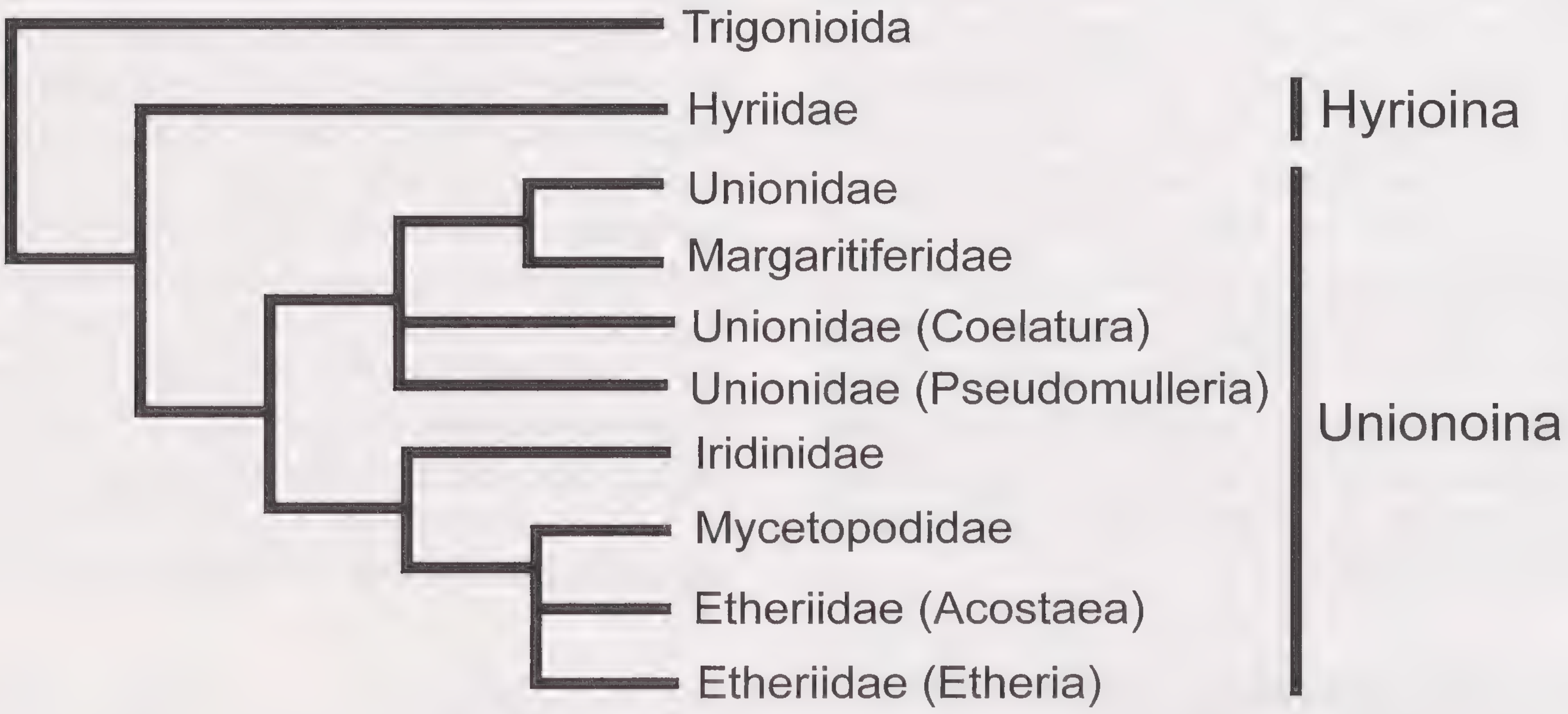

FIG. 2. Summary tree after the evolutionary relationships within the order Unionoida as portrayed in Bogan & Hoeh (2000: fig. 1) combined with those in Hoeh et al. (2001: fig. 14.4). Familial nomenclature: from Bogan & Hoeh (2000); subordinal nomenclature: proposed here.

MATERIALS & METHODS

We obtained G & C's aligned dataset from the following website: http://www.mussel-project.net/. GenBank accession numbers for all of the sequences analyzed herein are presented in G & C (Table 2) except for the *COI* sequences of *Mytilus edulis* (Hoeh et al., 1996) and *Astarte castanea* (GenBank accession number AF120662). To align the *Mytilus* and *Astarte COI* sequences with those in G & C's matrix, all *COI* DNA sequences were translated to amino acids, using the *Drosophila* mtDNA genetic code, and aligned with CLUSTAL_X (Larkin et al., 2007). Subsequently, the amino acid alignment was used to align the nucleotide sequences. The three-partition matrices utilized in our phylogenetic analyses, outlined below, are available upon request.

Phylogenetic trees were estimated using Bayesian inference (BI) and maximum parsimony (MP) approaches. Bayesian analyses were conducted using the program MrBayes (version 3.1.2; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Bayesian searches were run for 10 million generations with ten search chains, and the data were divided into five partitions (*COI*: 1st, 2nd and 3rd codon positions; *28S*; morphology/life history), saving 10,000 trees (one tree every 1,000 generations). These searches utilized the GTR + G + I substitution model (Rodriguez et al., 1990) for the DNA partitions and the standard discrete model (Lewis, 2001a; Ronquist et al., 2005) for the morphology/life history data partition. To allow each partition to have its own set of parameter estimates, *revmat*, *tratio*, *statefreq*, *shape*, and *pinvar* were all unlinked during the analysis. Burn-in was determined by visual inspection of the likelihood score plots obtained as the trees were written to the tree file. In the Bayesian analyses, stationarity was reached before one million generations, and the first 1,000 trees were discarded (i.e., the first million generations) as the burn-in.

Maximum parsimony searches were done with PAUP* (v.4.0b10; Swofford, 2002). Heuristic searches of the CE dataset were conducted with 10,000 random stepwise addition sequences with TBR branch-swapping, max trees set at 20,000, gaps handled as missing data, and MULTREES in effect. Additionally, 10,000 full heuristic non-parametric bootstrap replicates were done to assess levels of nodal support. All phylogenetic analyses were done on CE datasets that included the *Mytilus* and *Astarte COI* sequences and MP analyses were carried out on CE matrices with transformed (= 3rd codon position transitions were deleted) and non-transformed *COI* sequences. All MP analyses used equal weighting for each character in the CE matrix and all BI analyses used the CE matrix with non-transformed *COI* sequences. All of the phylogenetic analyses presented herein included the *COI* sequences from *Pseudomulleria, Coelatura* and *Obliquaria*.

The optimization of cemented (= oyster-like) vs. non-cemented shell morphology was based on the Bayesian topology with the highest overall posterior probability and was carried out using the ML algorithm in Mesquite (v.2.6; Maddison & Maddison, 2008). Both the Markov k-state one parameter model (MK1 model invokes equal forward and backward transition rates; Lewis, 2001a) and the "Asymmetrical Markov k-state 2 parameter model" (AsymmMK model in which "forward" and "backward" transition rates can be different; Pagel, 1997; Mooers & Schluter, 1999; Maddison, 2006) were explored for differences in optimization results and overall likelihood score. Because the two models are nested with the AsymmMK model having one more parameter than the MK1 model, a likelihood ratio test following a chi-squared distribution (df = 1) can be used to determine whether one model fits the data significantly better than the other (Pagel, 1994; see the *Mesquite* manual). The optimizations incorporated branch length and parameter estimates from the Bayesian analyses. To make decisions regarding the significance of ancestral character states, ancestral character state estimates with a log likelihood two or more units lower than the best state estimate (decision threshold [T] set to T = 2) were rejected (Edwards, 1972; Pagel, 1999). Generally viewed as a conservative cutoff, this threshold has been used by numerous recent authors (e.g., Moczek et al., 2006; Fernandez & Morris, 2007; Murphy et al., 2007; Koepfli et al., 2008).

RESULTS

Figures 3–5 contain the best (BI) and strict consensus (MP) trees produced by our analyses of the G & C CE dataset. Figure 3 portrays the best BI tree (= tree with the highest overall posterior probability) with parsimony-estimated branch lengths. Figure 4 portrays the strict consensus MP tree that resulted from analysis of the CE matrix containing transformed *COI* sequences and Figure 5 portrays the strict consensus MP tree that resulted from analysis of the CE matrix containing non-transformed *COI* sequences. Significant nodal support is indicated by posterior probabilities ≥ 0.95 (Fig. 3) and bootstrap percentages ≥ 70 (Figs. 4, 5).

Drastic topological effects were observed among the trees resulting from distinct analytical procedures (e.g., Figs. 3, 4 [from techniques that utilize corrections for *COI* sequence saturation] vs. Fig. 5 [no correction for saturation]). For example, Figures 3 and 4 display the Hyriidae as the basal unionoid bivalve lineage while Figure 5 indicates that that family is sister to the Etherioidea (*sensu* Bogan & Hoeh, 2000; Hoeh et al., 2001: Iridinidae + Mycetopodidae + *Acostaea*

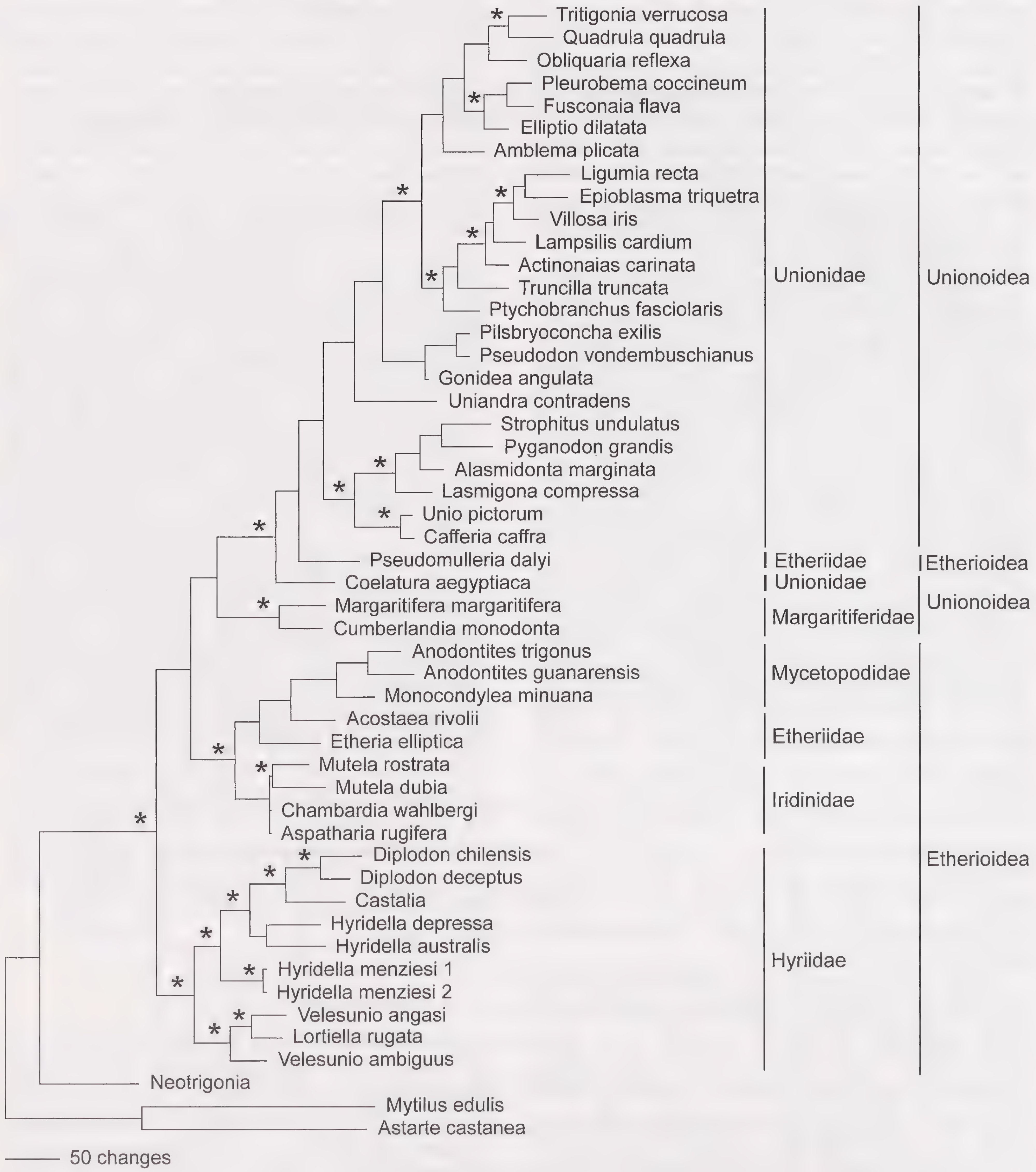

FIG. 3. Best tree obtained from 10 million generation Bayesian searches of the CE dataset with 10 search chains and five data partitions under the GTR + G + I model. Posterior probabilities ≥ 0.95 (from the BI majority-rule tree) are indicated by asterisks. Familial and superfamilial nomenclature: from G & C (2006).

+ *Etheria*) + *Pseudomulleria*. Furthermore, the MP phylogenetic analysis utilizing the CE matrix with non-transformed *COI* sequences produced a consensus tree with fewer clades possessing significant nodal support values (Fig. 5) compared to the trees generated using techniques that compensate for *COI* sequence saturation (Figs. 3, 4). For example, the Hyriidae was consistently found statistically monophyletic in the BI (Fig. 3) and "transformed *COI*" CE MP (Fig. 4) analyses, but this was not the case in the "non-transformed *COI*" CE MP analysis (Fig. 5).

The family Etheriidae (*sensu* G & C: unionoid bivalve oysters) is not a monophyletic taxon in any of the three trees generated herein (Figs. 3–5). The best BI tree (Fig. 3) clearly indicates, via topology and significant nodal support values, that *Pseudomulleria* is a unionid and that *Acostaea* and *Etheria* are members of a monophyletic Etherioidea (*sensu* Bogan &

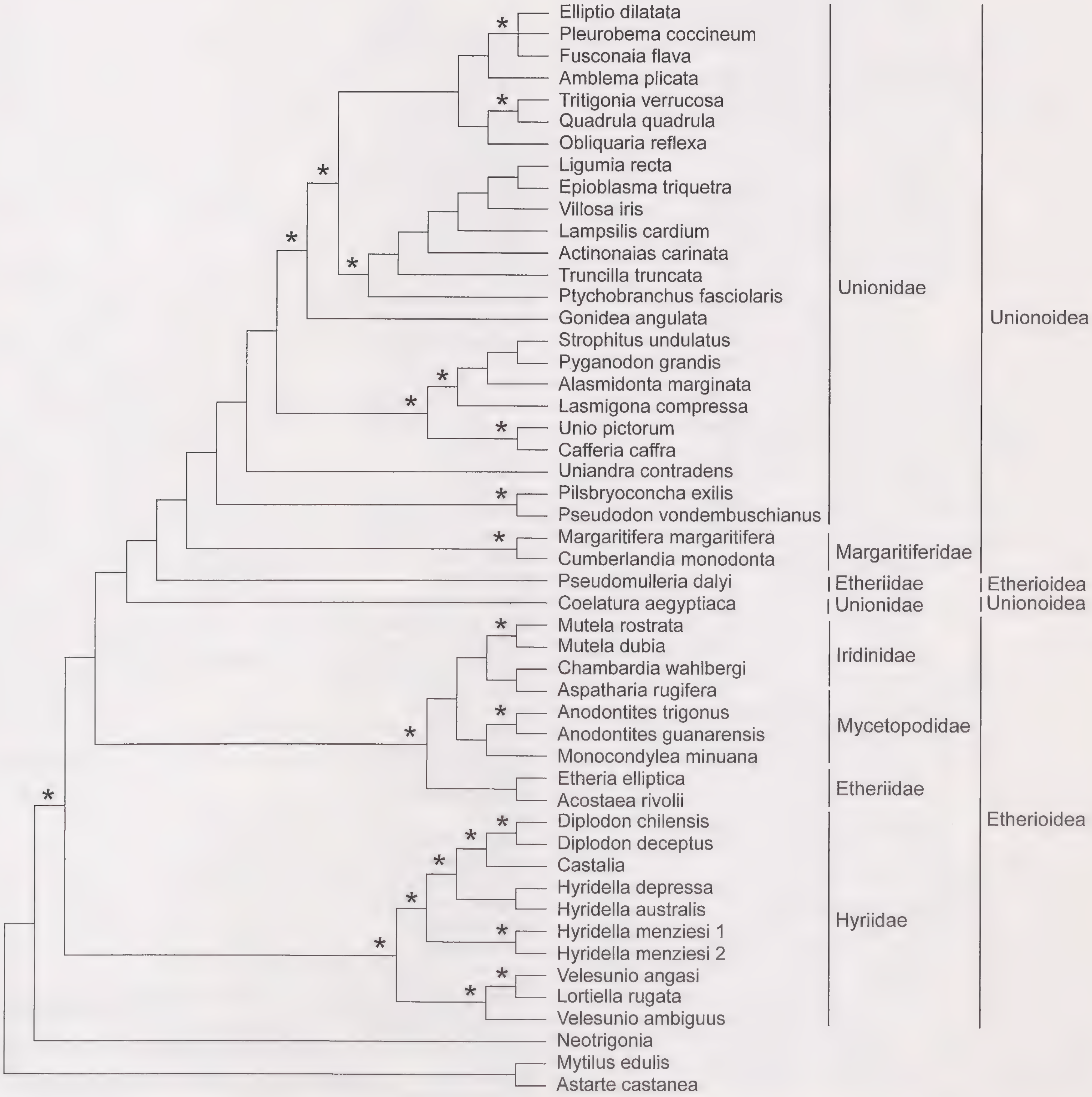

FIG. 4. Strict consensus of all equally parsimonious trees obtained by maximum parsimony analyses of the transformed CE dataset from 10,000 heuristic, random stepwise addition sequence replicates utilizing TBR branch swapping and 'max trees' set to 20,000. Bootstrap support values ≥ 70% (from 10,000 full heuristic bootstrap replicates) are indicated by asterisks. Familial and superfamilial nomenclature: from G & C (2006).

Hoeh, 2000; Hoeh et al., 2001). The Etherioidea (*sensu* Bogan & Hoeh, 2000; Hoeh et al., 2001) was also statistically supported as monophyletic by the analyses of the "transformed *COI*" CE matrix (Fig. 4) and "non-transformed *COI*" CE matrix (Fig. 5). In addition, there was a significant divergence among phylogenetic techniques regarding the evolutionary reality of the largest unionoid bivalve family: the Unionidae (including *Pseudomulleria*) was found monophyletic (Fig. 3) and, alternatively, non-monophyletic (Figs. 4, 5). However, only the monophyly indicated in Figure 3 was supported by a significant nodal support value.

A ML optimization of cemented (= oyster-like) vs. non-cemented shell morphology, using the AsymmMK model of character evolution, is presented in Figure 6. Although the AsymmMK model had a slightly higher log-likelihood score, the likelihood ratio test showed that this model did not fit the data significantly better than the MK1 model (equal forward and backward

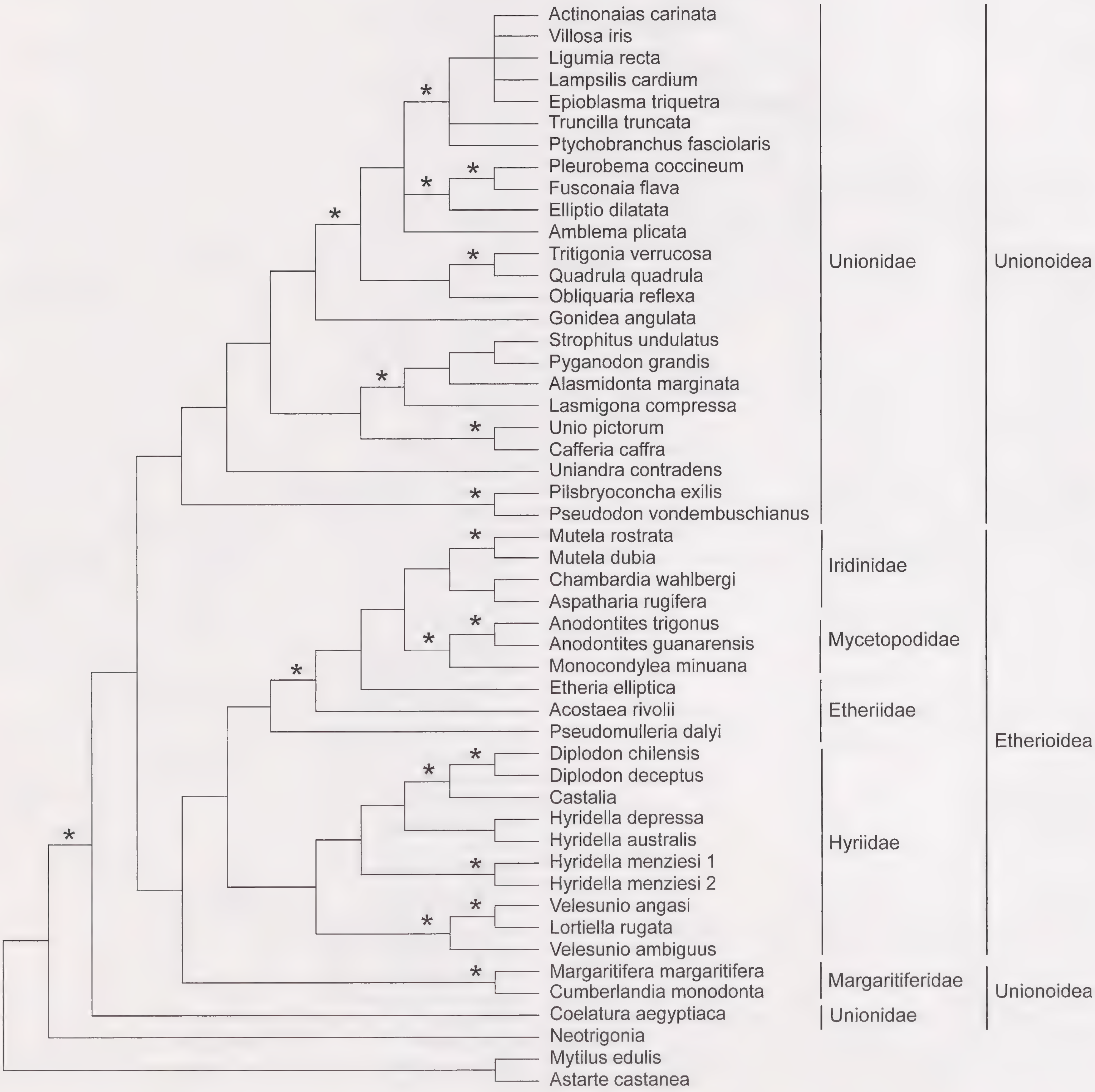

FIG. 5. Strict consensus of all equally parsimonious trees obtained by maximum parsimony analyses of the non-transformed CE dataset from 10,000 heuristic random stepwise addition sequence replicates utilizing TBR branch swapping and 'max trees' set to 20,000. Bootstrap support values ≥ 70% (from 10,000 full heuristic bootstrap replicates) are indicated by asterisks. Familial and superfamilial nomenclature: from G & C (2006).

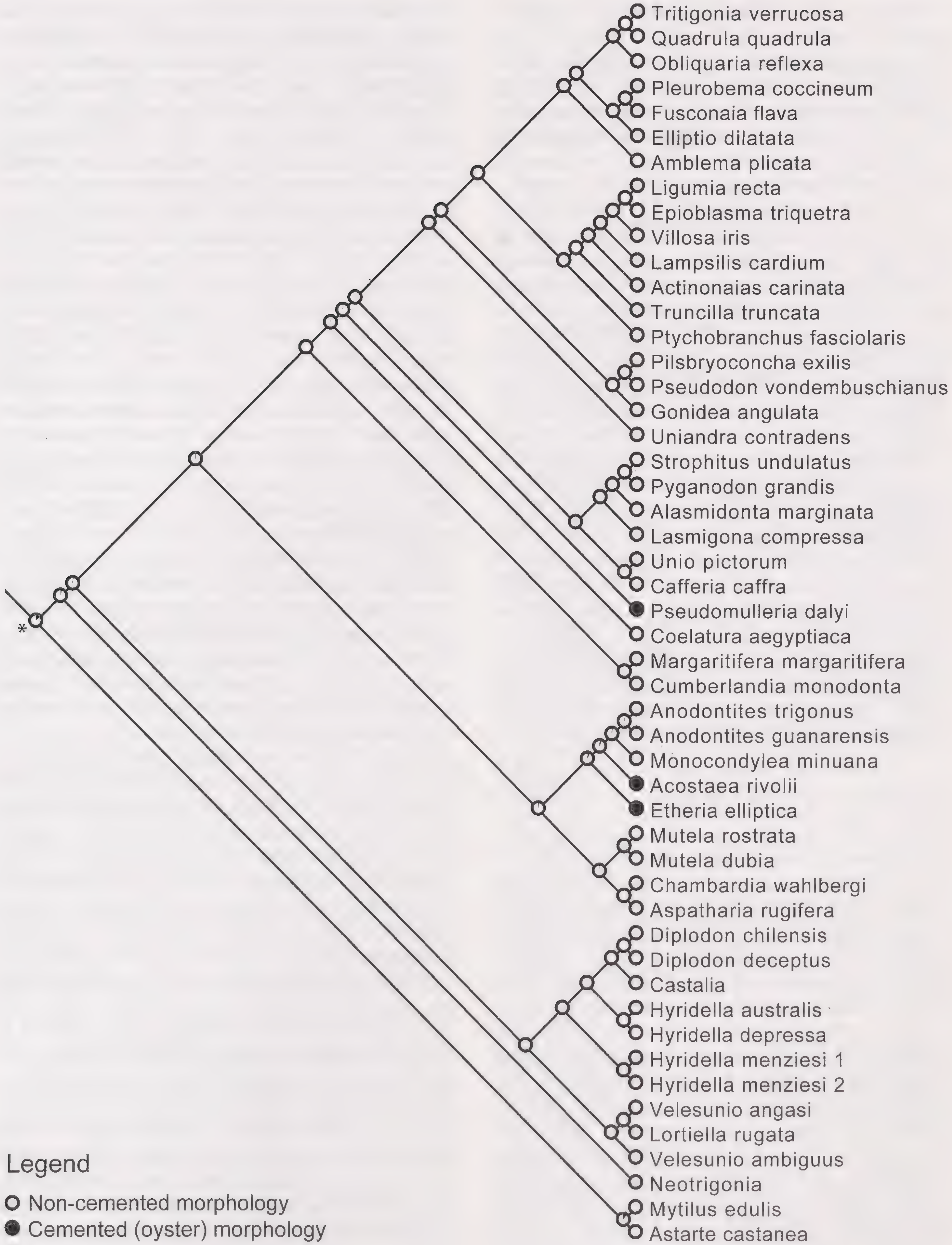

Fig. 6. Maximum likelihood optimization of cemented vs. non-cemented shell morphology, on the tree in Figure 3, produced by Mesquite using the AsymmMK model. Significance of ancestral character state estimates determined by one character state having a log likelihood two or more units higher than the other state. All nodes are significant for a single character state except for the single node denoted with an asterisk (*).

transition rates). However, optimizing with either model resulted in the same inference: three independent transitions from non-cemented to cemented morphology. Using the AsymmMK model, all of the ancestral character state estimations were significant except for the root node (as indicated by an asterisk on Fig. 6), whereas all nodes were significant under the MK1 model.

The BI analysis of G & C's CE matrix utilized the most sophisticated methodologies for dealing with inter- and intra-partition rate heterogeneity and produced the most resolved trees (based on comparisons of tree topologies). The tree produced by BI analysis (Fig. 3) indicates significant support for the monophyly of the Unionidae (including *Pseudomulleria*), Margaritiferidae, Hyriidae, Etherioidea (excluding both *Pseudomulleria* and the Hyriidae [addressed below]) and Unionoida. In two of our three trees (Figs. 3, 4), the Hyriidae is not the sister lineage to the Etheriidae (*sensu* G & C) + Iridinidae + Mycetopodidae clade as portrayed in the preferred tree of G & C (Fig. 1). Only MP analysis of G & C's CE matrix containing non-transformed *COI* sequences produced a clade containing hyriids plus Etheriidae (*sensu* G & C) + Iridinidae + Mycetopodidae (Fig. 5). However, few of the estimated interfamilial relationships are supported by significant nodal support values in our analyses of G & C's CE matrix (Figs. 3–5).

DISCUSSION

Many recent authors view analyses of concatenated multi-gene datasets as those which produce the best possible estimates of phylogeny (e.g., Sanderson et al., 2003; Driskell et al., 2004; Gadagkar et al., 2005; de Queiroz & Gatesy, 2007). Therefore, it is our opinion that future combined evidence analyses of palaeoheterodont bivalve phylogeny should not exclude data unless a compelling case can be made for each exclusion. For example, previous phylogenetic analyses have deleted "extremely long branch" terminals because of significant variation in estimated terminal branch lengths (e.g., Spears & Abele, 2000). However, this problem can be better addressed by adding taxa to break up long branches (Graybeal, 1998), thus eliminating the need for discarding terminals. Additionally, if particular sequences are objectively found to be "inferior" to others (e.g., due to sequencing ambiguities such as the presence of many uncertain base calls, single nucleotide indels in protein coding genes or specimen identification/laboratory processing errors), this could constitute a valid reason for exclusion. However, strict and objective criteria need to be employed in making this type of determination to guard against potential investigator bias in matrix construction (e.g., Hillis, 1998). After all is said, "By combining [datasets], the results can be interpreted in a broader context, and the shortcomings and advantages of each partition can be assessed relative to the others." (G & C, p. 347) still rings true. We think that a model paper in this regard is Campbell et al. (2005), which presented analyses of a three partition data matrix containing all of the relevant DNA sequences and subsequently discussed potential problems with individual sequences (but its figured trees were based on analyses that included all relevant sequences). It is also our opinion, and that of others (e.g., Huelsenbeck & Ronquist, 2001; Lewis, 2001b; Ronquist & Huelsenbeck, 2003; Huelsenbeck et al., 2004; Lewis et al., 2005; Alfaro & Holder, 2006), that phylogenetic methodologies that attempt to compensate for saturation should be employed, in parallel, if an investigator desires to use equally weighted MP analyses on non-transformed DNA sequences. Again, Campbell et al. (2005) is a model paper in that it used both BI and equally weighted MP analyses on non-transformed DNA sequences.

Regarding the validity of the Etheriidae (= *Etheria* + *Acostaea* + *Pseudomulleria; sensu* G & C), Graf's (2000) determination of sister taxa status for *Acostaea* and *Etheria*, based on an analysis of morphological and life history characters, is consistent with a monophyletic Etheriidae, but *Pseudomulleria* was not included in that analysis. G & C, whose preferred analyses contained a *Pseudomulleria* terminal but only included morphological and life history characters in the matrix, confirmed the monophyly of the Etheriidae. Alternatively, Bogan & Hoeh's (2000) analysis of *COI* DNA sequences, that included data for *Etheria*, *Acostaea* and *Pseudomulleria*, indicates that *Pseudomulleria* is a unionid, as does Woodward's (1898) anatomical study, thus rejecting the concept of a monophyletic Etheriidae. Herein, phylogenetic analyses of the G & C CE dataset also produced best BI and strict consensus MP trees that statistically rejected etheriid (*sensu* G & C) monophyly (Figs. 3–5). Thus, all topologies from the total evidence dataset analyzed herein are consistent in that they do not support etheriid monophyly but, alternatively, Figures 3 and 4 do support Prashad's (1931) and Heard & Hanning's (1978) independent origins/convergence hypothesis for "etheriid" conchology. Regarding the latter, ML-based optimization of cemented (= oyster-like) vs. non-cemented shell morphology, using the BI tree (Fig. 3), indicates three distinct origins of cementation in the Unionoida (Fig. 6; as did the parsimony-based optimization presented in Bogan & Hoeh [2000: fig. 1]). The above discussion indicates that G & C's concept of the Etheriidae requires revision.

Regarding the interfamilial evolutionary relationships of the Hyriidae, classifications of Ortmann (1912, 1921), the morphology-based analyses of Graf (2000), Hoeh et al. (2001), and Roe & Hoeh (2003), the total evidence-based analysis of Roe & Hoeh (2003), as well as the preferred CE analysis of G & C (Fig. 1) all support the hypothesis of a relatively close evolutionary relationship among hyriid and etherioid (*sensu* Bogan & Hoeh, 2000; Hoeh et al., 2001) bivalves. Alternatively, the *COI* sequence- and total evidence-based analyses of Bogan & Hoeh (2000), Hoeh et al. (2001, 2002) and Walker et al. (2006) indicate that the Hyriidae is the basal unionoid bivalve lineage. Analyses of G & C's CE dataset, using phylogenetic methods that compensate for the acknowledged saturation in *COI* sequences at this time depth (Hoeh et al., 1998; Bogan & Hoeh, 2000; Graf & Ó Foighil, 2000; Graf & Cummings, 2006), produce best BI and strict consensus MP trees that support the latter hypothesis (Figs. 3, 4). The above discussion suggests that G & C's concept of the Etherioidea requires revision. Within the confines of the analytical methods employed herein, which are clearly not exhaustive, the only tree to support the Etherioidea *sensu* G & C (Fig. 5) required for its generation an equally weighted MP analysis that used non-transformed *COI* sequences. We predict that phylogenetic methods that correct for saturation in DNA sequences (e.g., BI and "transformed" MP analyses) will typically yield better estimates of unionoid bivalve phylogeny than will methods that do not correct for saturation. However, the generally poor nodal support values for most interfamilial relationships obtained herein from all analyses of G & C's CE dataset (Figs. 3–5) indicate that we cannot objectively choose among interfamilial relationship hypotheses at this time. Thus, robust estimates of unionoid bivalve (1) interfamilial relationships, (2) character evolution, and (3) lineage-specific species richness, as well as a stable, phylogeny-based classification for the group, await appropriate phylogenetic analyses utilizing more inclusive datasets.

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THE GENUS *POSTICOBIA* (MOLLUSCA: CAENOGASTROPODA: RISSOOIDEA: HYDROBIIDAE S.L.) FROM AUSTRALIA AND NORFOLK ISLAND

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ABSTRACT

The freshwater gastropod genus *Posticobia* Iredale, 1943, is found predominately in eastern Australia and is redefined using morphological and anatomical characters. The genus is found in a wide range of habitats from isolated springs in central Australia to the large coastal rivers and lakes of New South Wales and Queensland. The genus as currently recognised is considered to contain three species, *Posticobia brazieri* (E. A. Smith, 1882) from eastern Australia, ***Posticobia ponderi*** n. sp. from Mulligan Springs, South Australia, and *Posticobia norfolkensis* (Sykes, 1900) from Norfolk Island.

Key words: Hydrobiidae, *Posticobia*, anatomy, taxonomy, morphology, Australia.

INTRODUCTION

The dominant group of freshwater gastropod molluscs in Australia is the Hydrobiidae, with over 180 currently named taxa (Smith et al., 2002). (The use of this family name for the Australasian taxa is currently under review and is here used in the broad sense, following Ponder et al., 2008.) However, based on the range of forms present in Australian museum collections and unpublished genetic data, this figure represents only a fraction of the true Australian hydrobiid fauna. This highly speciose group can be found in virtually all freshwater habitats, from small high mountain streams (Clark et al., 2003) to isolated springs in the arid interior (Ponder et al., 1996) to caves in southern Tasmania (Ponder et al., 2005). The bulk of the known species are narrow range endemics (Harvey, 2002; Ponder & Colgan, 2002), with small to very small distributions and live in habitats that do not allow easy access for such passive dispersal agents as birds or fish. Consequently, there is a tendency towards local endemism; examples include *Beddomeia* Petterd, 1889, *Fluvidona* Iredale, 1937, and *Austropyrgus* Cotton, 1942 (Ponder et al., 1993; Miller et al., 1999; Clark et al., 2003). On the other hand, genera that do live in such relatively open and therefore accessible habitats as coastal rivers and lakes tend to have few species with large distributions; examples include *Littoridinops* Pilsbry, 1952, *Hydrobia* Hartmann, 1821 (Thompson, 1999; Wilke & Davis, 2000), and one of the species dealt with herein.

Posticobia has a wide, largely coastal distribution in the eastern half of Australia (Fig. 1), inhabiting freshwater springs, streams, rivers, and lakes down to the freshwater-brackish water interface. It occurs from the Northern Territory, east across northern Queensland and south to Sydney, central New South Wales, with scattered populations in southeastern South Australia and isolated populations in western Victoria, northeastern South Australia, and western Queensland. Outside of the Australian mainland, the genus has been recorded from Norfolk Island, with *Posticobia norfolkensis* (Sykes, 1900). However, this form now appears to be extinct (Ponder, 1981), and along with *Beddomeia tumida* Petterd, 1889, from Tasmania (Ponder et al., 1993), have the dubious distinction of being the only named Australian hydrobiids to be reported as extinct.

Posticobia is typically very abundant and can reach extremely high densities, sometimes in the order of 100,000s per square metre, as was observed in the Wilson River at Banglow Rd., N of Lismore in 1993. When extremely high population densities were observed, the streams had very high water quality with intake riparian vegetation. Therefore, when very high densities are present, it appears to be a good indicator of stream health. However, very little is known of the biology of *Posticobia*, or of most other Australian hydrobiids. Casual observations, however, suggest that *Posticobia brazieri* may breed throughout much of the year due to the presence of egg capsules and juveniles of various sizes at different times of the year, and because their reproductive systems usually appear to be mature irrespective of the season.

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The taxonomy of the genus is typical of the early reliance on shell characters as a means of classification. Smith (1882) named a distinctive species of freshwater snail collected by John Brazier from the Clarence River, at Grafton, northern New South Wales, as *Hydrobia brazieri*. Subsequently, *Amnicola positura* from Lismore, northern New South Wales, was proposed (Petterd, 1884). No further forms were named until 1943, when Iredale considered *Hydrobia brazieri* to be so distinctive in shell shape and in the possession of a keel on the last whorl, that he erected the genus *Posticobia*. Further, he named an additional species, *Posticobia chena*, from the stomach of a duck shot at Chichester Dam, near Dungog, central New South Wales. *Paludestrina norfolkensis* Sykes, 1900, from Norfolk Island (approximately 1,650 km ENE of Sydney), was included by Ponder (1981) in the *Posticobia* on the basis of the similarity of its shell, opercular, and radular characters to those of *Posticobia brazieri*. Ponder & Clark (1990) suggested *Posticobia* was a subgenus of the widespread genus *Austropyrgus* Cotton (= *Fluvidona* Iredale, as it was then recognised). Smith (1992) listed *Posticobia* as a full genus with two valid species *P. brazieri* and *P. norfolkensis*. A recent molecular study (Perez et al., 2005) suggested that *Posticobia* is a valid genus sister to *Austropyrgus*.

The purpose of the present study was to clarify the taxonomic status of *Posticobia* and to determine whether the morphological variation exhibited by *Posticobia* represents a single widespread variable species or a complex of several species using shell, radula and anatomical characters.

MATERIALS AND METHODS

Collection

Specimens used for anatomy and allozymes were collected between May 1992 and October 1994 by washing specimens from the surface of the substratum, such as stones, wood or roots, and using a hand sieve to sweep aquatic vegetation along the edges of springs, small streams, rivers and lakes. Where samples allowed, part was frozen and the rest was fixed in 10% formalin buffered with sodium bicarbonate and then transferred to 5% buffered formalin. Samples were obtained from across the mainland range of *Posticobia* (Fig. 1). However, attempts to recollect specimens from western Victoria were unsuccessful.

Subsamples of specimens were measured, dissected and mounted for SEM using standard protocols (e.g., Ponder et al., 1989; Clark et al., 2003). The material used is housed in the institutions listed below under abbreviations. A discriminant analysis based on the shell measurements (SH, SW, AH, BW, CV, TW) was run using MYSTAT 12 ver. 12.02 2007 (SYSTAT Software Inc., Richmond, California, USA).

Allozyme Electrophoresis

Twenty populations (Clark, 1997) were examined electrophoretically using Titan III cellulose acetate plate electrophoresis and were scored for 21 loci. Clark (1997) listed the loci examined, the running conditions and the alleles observed for each population. Results were analysed with BIOSYS-1 (Swofford & Selander, 1981, 1989). However, due to the small sample sizes for most of the populations examined, the question as to how many species may be represented was poorly resolved. Therefore, these results are not presented here.

ABBREVIATIONS

Collections: AMS – Australian Museum, Sydney, New South Wales, Australia (material listed from AMS has the prefix C.); MV – Museum of Victoria, Melbourne, Victoria, Australia; NMNZ – National Museum of New Zealand, Wellington, New Zealand; NHML, The Natural History Museum, London, England; QM – Queensland Museum, Brisbane, Australia; SAM – South Australian Museum, Adelaide, South Australia, Australia.

Shell and Opercular Measurements: AH – aperture height; BW – body whorl height, CV – convexity ratio; OL operculum length; OW – operculum width; OSL – opercular smear length; OSW – opercular smear width; SH – shell height; SW – shell width, TW – number of body whorls.

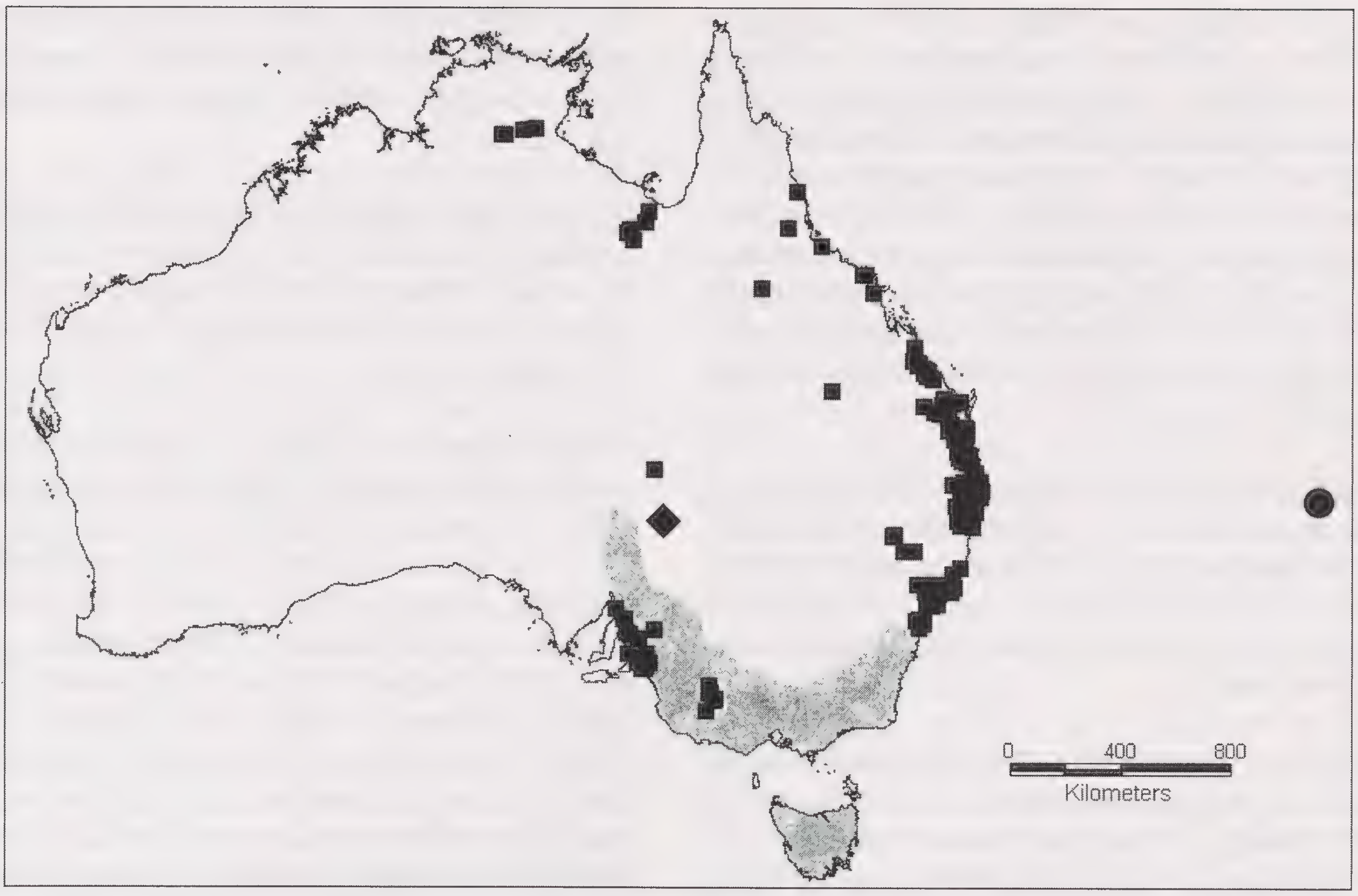


FIG. 1. Distribution of *Posticobia*. ■ – *P. brazieri*; ◆ – ***P*. ponderi**; ● – *P. norfolkensis*. The shaded area represents the known range of *Austropyrgus*.

Anatomical Figures: ag – albumen gland; bc – bursa copulatrix; bd – bursal duct; ca – caecum; cg – capsule gland; co – coiled oviduct; dgo – digestive gland opening; go – genital opening; in – intestine; os – oesophagus; pe – penis; pg – prostate gland; pw – pallial wall; sd – seminal receptacle duct; sr – seminal receptacle; ss – style sac; st – stomach; vc – ventral channel; vd – vas deferens.

Geographic Terms: Ave. – Avenue; ca. – approximately; Cr. – Creek; Dr. – Drive; Hwy. – Highway; Mt. – Mount; NP – National Park; Rd. – Road; Ri. – River; SF – State forest.

TAXONOMY

Family Hydrobiidae
Subfamily Tateinae

Classification follows Bouchet & Rocroi (2005) and Ponder et al. (2008).

Genus *Posticobia* Iredale, 1943

Posticobia Iredale, 1943: 204. Type species: *Hydrobia brazieri* Smith, 1882, by original designation.

Diagnosis

Shell ovate to broadly conical; sutures slightly to well impressed; body whorl convex to strongly keeled, smooth. Operculum flat, paucispiral, yellow, with a white smear and 2–5 (typically 2–3) weakly developed pegs on inner surface. Radula with 3–4 (typically 4) basal cusps on central teeth. Penis simple, tapering; prostate gland closed, more or less kidney-shaped, with pallial vas deferens emerging from just before pallial wall on ventral side. Female genital system with simple, inverted U-shaped proximal coiled oviduct and short distal coiled oviduct with single bend. Pallial oviduct with opening overlapping anterior end of capsule gland.

Description

Shell – Shell small to medium-sized for family, 1.37–3.31 mm in height, 1.18–2.82 mm in width, ovate to broadly conical in shape; spire of average height, outline convex; sutures slightly to well impressed. Aperture 0.68–1.52 mm in height, oval, not disjunct from last whorl. Teleoconch of 2.25–4.30 convex whorls, body whorl 1.08–2.42 mm in height, last whorl evenly convex to sub-shouldered, keel absent to well developed. Teleoconch sculpture smooth, with fine to coarse growth lines. Inner lip of aperture firmly adhering to parietal wall to slightly separated from parietal wall. Outer lip weakly thickened to heavily thickened, lacking external varix, not reflected. Umbilicus partially open to narrow in adults, narrowly open in juveniles. Colour opaque, yellowish-brown, periostracum thin, may impart darker colour. Protoconch of about 0.85–1.30 whorls; sculptured with sparsely to densely distributed irregular shallow pits.

Operculum – Ovate, thin, flat, yellow, horny, paucispiral, with eccentric nucleus, 0.65–1.47 mm in length, 0.51–1.06 mm in width. White smear 0.06–0.57 mm in length, 0.03–0.40 mm in width; up to 5 weakly developed pegs on inner side.

Radula – Typical of subfamily. Central teeth with 4–5 lateral cusps, median cusp of medium width, sharply pointed and about 2 times longer than adjacent cusps; 3–4 pairs of basal cusps; basal tongue U-shaped, just protruding past lateral edge. Lateral teeth: with 4–5 cusps on each side, medium cusp of medium width, sharply pointed and about 2 times longer than adjacent cusps; basal projection U-shaped, lateral flange longer than cutting edge. Inner marginal teeth with 21–31 cusps, on distal and outer sides of each tooth, inner side smooth. Outer marginal teeth with 25–35 cusps, on distal and inner sides of each tooth, outer side smooth.

External Features – Typical of subfamily. Animal pigmentation variable, from weakly to well developed (grey to black), cephalic tentacles long, slender, slightly tapering, with narrow, median-dorsal unpigmented line (corresponding to line of dorsal cilia). Snout with some cilia dorsally, with narrow, well-developed band laterally and latero-dorsally just behind labia. Tentacles with well-developed, mid-dorsal and two ventro-lateral strips of cilia.

Mantle Cavity – Typical of subfamily. Ctenidium with 19–32 triangular filaments (width at widest point 0.12–0.39 mm), apices near right side, posterior end abutting pericardium. Osphradium 0.22–0.66 mm in length, white, elongately oval, located between posterior end and middle of ctenidium. Hypobranchial gland inconspicuous to well developed, partly to completely covering rectum. Rectum straight or with weakly developed arch (arch more developed in males). Visceral coil: Renal organ (as mainly seen from renal gland) extends forward ca. 1/3–1/2 into pallial cavity, orientated longitudinally. Renal gland 0.28–0.72 mm in length and 0.16–0.34 mm in width. Pericardium more than 1/2 in pallial roof.

Stomach – Similar to other members of subfamily. Stomach 0.33–0.74 mm in length, style sac 0.22–0.67 mm in length with a very small to small gastric caecum, 0.02–0.17 mm in length.

Male Genital System – Similar to other members of subfamily. Testis occupying about 1.0–1.5 whorls. Seminal vesicle beneath anterior 1/4 to 1/2 of first whorl of testis behind stomach, loosely to tightly coiled over stomach, conspicuously coiled on both digestive gland and testis behind stomach. Prostate gland 0.40–0.73 mm in length, 0.17–0.34 mm in width, about 1/3 to 2/3 in pallial roof, kidney-shaped, oval to compressed-oval in section. Pallial vas deferens flush with surface of neck, slightly to strongly undulating from prostate gland to base of penis. Penis unpigmented, distal part short and tapering, base broad, creased (when at rest). Penial duct strongly undulating in base, straight in distal portion of penis. Penis located just to right of midline, 0.22–0.56 mm behind right eye.

Female Genital System – Similar to other members of subfamily. Ovary simple, occupying about 0.75–1.40 whorls. Coiled oviduct with initial U-shaped bend orientated obliquely backwards; initial loop high to medium; proximal part simple; distal part straight or with one bend distal to seminal receptacle. Coiled oviduct and bursal duct joining just behind pallial wall with oviduct joining bursal duct from right side. Oviduct straight, before joining with bursal duct. Bursal duct 0.04–0.12 mm in length, 0.07–0.15 mm in width, parallel sided, straight. Seminal receptacle located on or near anterior edge of bursa copulatrix to towards middle of inner wall of bursa copulatrix. Bursa copulatrix 0.22–0.55 mm in length, 0.16–0.33 mm in width. Seminal receptacle 0.09–0.22 mm in length, 0.06–0.13 mm in width, pyriform in shape to a narrow sac with short duct. Rectum overlapping both albumen and capsule glands. Albumen gland 0.22–0.51 mm in length, 0.22–0.43 mm in width, about 1/2 to 2/3 of albumen gland in front of posterior pallial wall. Capsule gland 0.31–0.90 mm in length, 0.18–0.35 mm in width, with anterior end tapering to blunt, oval to compressed-oval in section, with distinct glandular zones. Ventral channel indistinct to distinct, with indistinct to moderate sized muscular vestibule. Genital opening small to medium in length, overlapping anterior end of capsule gland.

Egg Capsules – Egg capsules small, 0.30–0.40 mm in diameter, hemispherical in shape. They contain a single egg and are usually a greenish-yellow colour. They are found attached to various substrata, such as sand grains and vegetation or, rarely, attached to the shell of other individuals or other molluscs. They have been found in most samples collected during the length of this project.

Distribution

Posticobia is distributed in the eastern half of Australia and Norfolk Island (Fig. 1); it is found predominately in the streams and rivers flowing east along the coastal ranges, with a number of scattered populations found in parts of inland New South Wales, Victoria, Queensland, Northern Territory, and South Australia. *Posticobia* is found in a wide range of aquatic habitats from coastal lakes, such as the Myall Lakes of central New South Wales, coastal rivers and streams to isolated desert springs, such as Mulligan Springs in northern South Australia. There appears to be a large gap in distribution from Sydney south to Victoria west of Melbourne, where *Posticobia* appears to be replaced by the closed related genus *Austropyrgus* Cotton, 1942 (Clark et al., 2003; Perez et al., 2005). Where *Posticobia* occurs it is normally abundant and can be found crawling on all substrata, such as aquatic plants, wood, sand, gravel and rocks. *Posticobia* can be found sympatrically with *Fluvidona* Iredale, 1937, and *Jardinella* Iredale & Whitley, 1938, in northern New South Wales and southern Queensland, *Austropyrgus* in New South Wales and South Australia, *Tatea* Tenison Woods, 1879, and *Ascorhis* Ponder & Clark, 1988, in the Myall Lakes New South Wales.

Remarks

Posticobia is a member of a group of Australasian genera related to the estuarine genus *Tatea* Tenison Woods, 1879. Since its original description, *Posticobia* has been treated as a valid genus (Iredale, 1943, 1944; Ponder, 1981; Smith, 1992) or as a subgenus of *Fluvidona* of authors (= *Austropyrgus*) (Ponder & Clark, 1990). Some of the similarities that *Posticobia* shares with *Tatea* and related genera are 3–4 pairs of basal cusps on the central tooth, the presence of pegs (albeit rudimentary in *Posticobia*) on the inner surface of the operculum, and a simple, tapering penis.

Posticobia differs from *Fluvidona* in a number of characters, including its shell shape (pupiform in *Fluvidona*), the possession of a gastric caecum (lacking in *Fluvidona*), the oviduct joins the bursal duct ventrally (dorsally in *Fluvidona*), the yellow colour of the operculum (red in *Fluvidona*), and the poorly developed opercular pegs (well developed in *Fluvidona*). Allozymically the two genera are very different, with 17 fixed differences separating the two (Clark, 1997). *Posticobia* differs from *Austropyrgus* by its shell shape (pupiform to elongated conical), the opercular pegs are poorly developed (well developed in *Austropyrgus*), and the coiled oviduct is positioned just on the anterior edge of the bursa copulatrix near the albumen gland (it lies across more than half the lower portion of the bursa in *Austropyrgus*). Allozymically the two genera are more closely related to one another than either are to *Fluvidona*, with four fixed differences separating the two (Clark, 1997). *Posticobia* can be easily distinguished from other Australasian hydrobiid genera by its general shell shape, although there is some superficial resemblance to some species of *Beddomeia* Petterd, 1889, which occur only in Tasmania, outside the range of *Posticobia*. *Jardinella* differs from *Posticobia* in a number of characters including its shape, having only two pairs of basal cusps on the central tooth and having a no pegs or only a white smear present on the inner side of the operculum.

Posticobia can be found from slightly brackish waters, such as those of the Myall Lakes to small isolated springs in arid areas of South Australia and Queensland. The New Zealand hydrobiid genus *Potamopyrgus* Stimpson, 1865, shows similar wide tolerances from freshwater to brackish water (Haase, 2008).

Posticobia brazieri (E. A. Smith, 1882)
Figures 1–4, 7–10, 13, 14, 17–20, 23, 24, 27–35

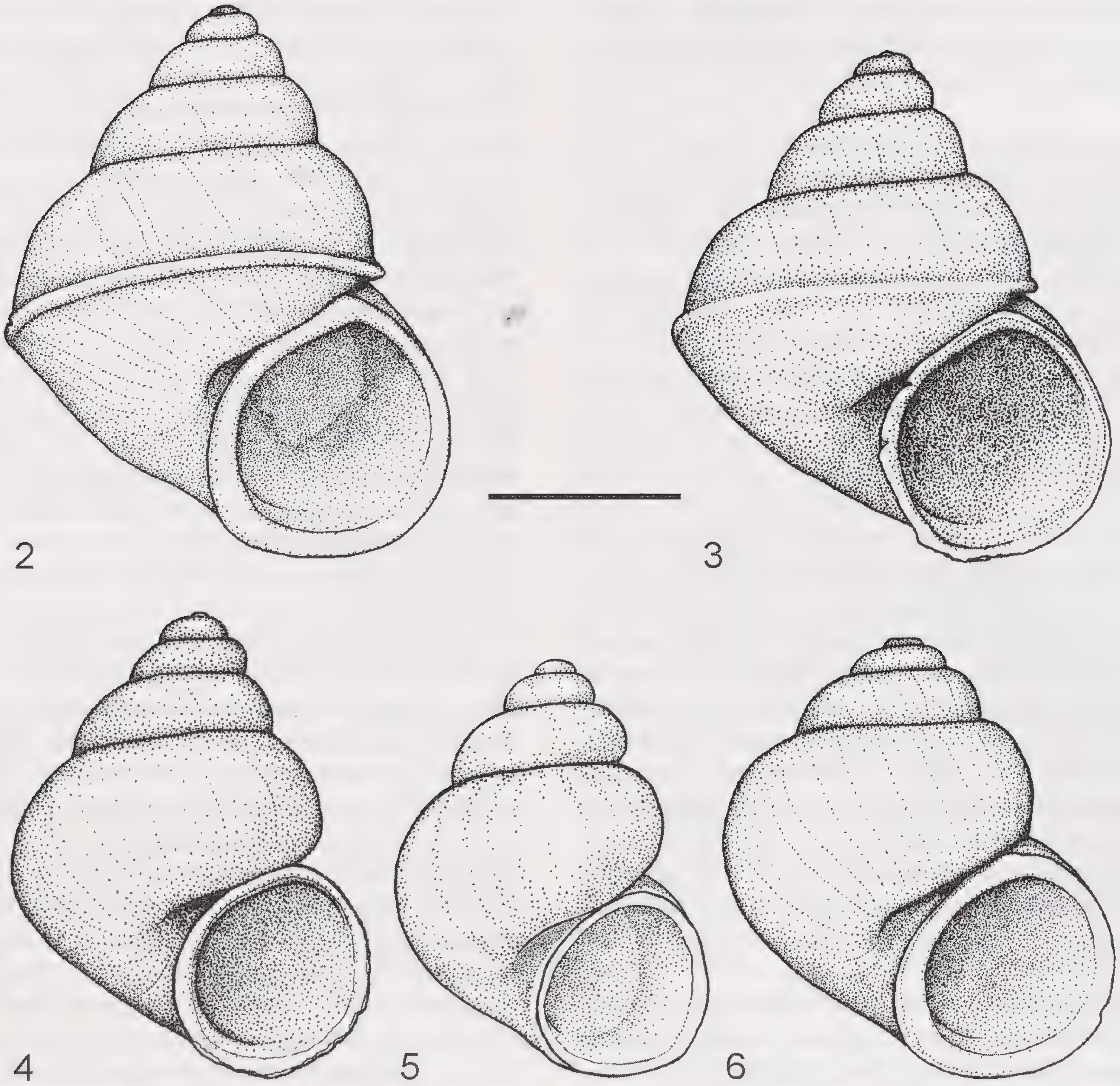
Hydrobia brazieri E. A. Smith, 1882: 269, pl. 7, fig. 21; Brazier, 1889: 72; Hedley & Musson, 1892: 563.

Amnicola positura Petterd, 1884: 159.

Amnicola carinata Brazier, 1889: 72 (*nom. nud.*).

Petterdiana brazieri – Hedley, 1904: 184; Hedley, 1915: 716.

Posticobia chena Iredale, 1943: 204; Iredale, 1944: 115, text-fig.



FIGS. 2–6. *Posticobia* species. FIGS. 2–4: *P. brazieri*; FIG. 2: Syntype of *Hydrobia brazieri* (BMNH 1879.5.21.369-378); FIG. 3: Syntype of *Amnicola positura* (C.19919); FIG. 4: Syntype of *Posticobia chena* (C.100582); FIG. 5: Holotype of ***P. ponderi*** (C.461157); FIG. 6: *P. norfolkensis*; Syntype of *Paludestrina norfolkensis* (BMNH 1856.10.27.94). Scale bar = 1 mm.

Posticobia brazieri – Iredale, 1943: 204; Iredale, 1944: 115; Ponder, 1981: 17, 18, fig. 2, 6–8; Smith, 1992: 53, 54; Ponder et al., 2000.
Fluvidona (*Posticobia*) cf. *brazieri* – Ponder & Clark, 1990: 348.

Type Material

Syntypes NHML 1879.5.21.369-378 (65); syntypes NHML 1886.12.3.36-45 (15), in G. A. Angas Collection.

Type Locality

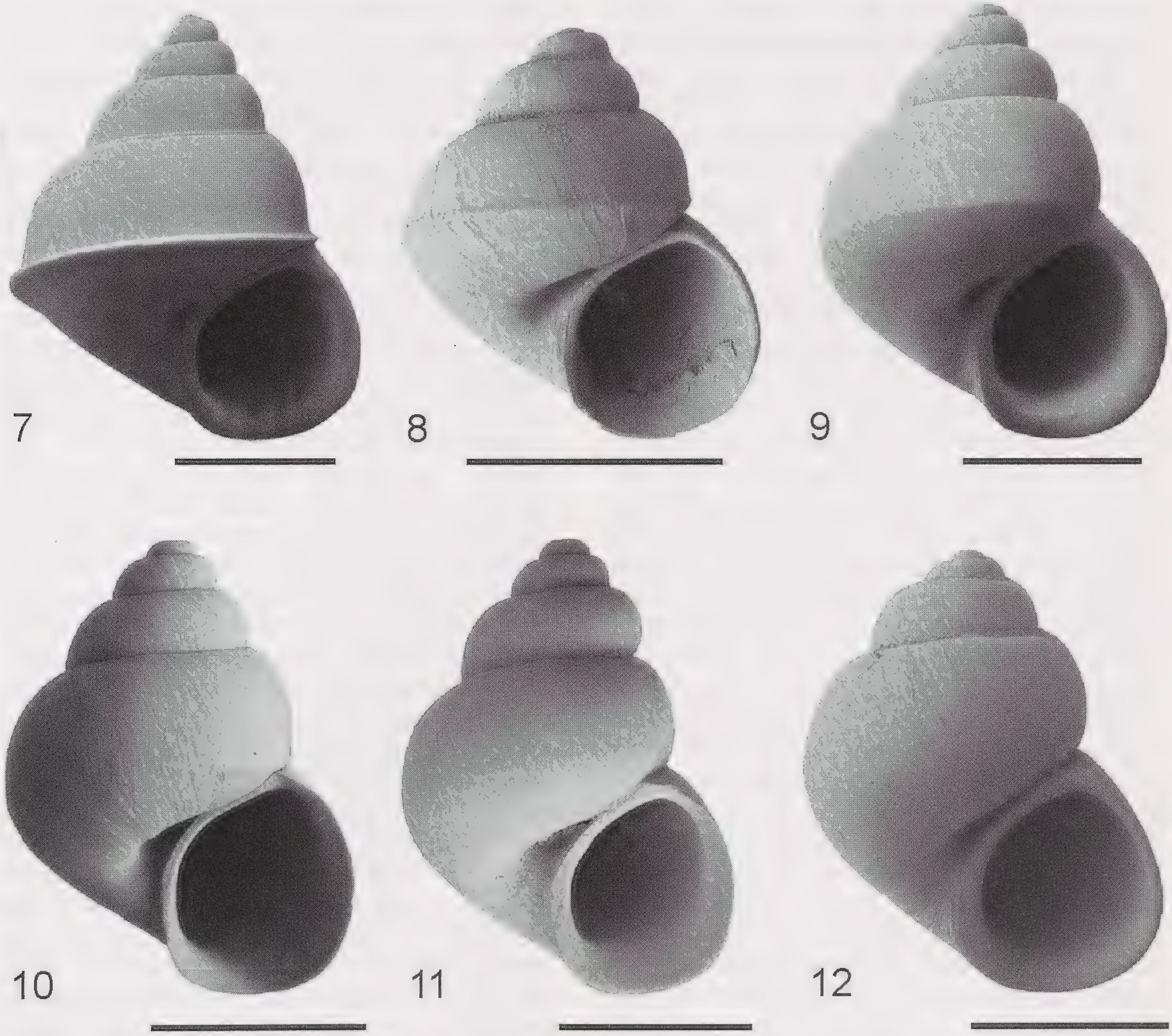
In freshwater creek, Clarence Ri., South Grafton, New South Wales, Australia, 1879, J. Brazier.

Type Material of Synonyms

Amnicola positura, syntypes C.19919 (4), Richmond Ri., near Lismore, NSW, W. F. Petterd; *Posticobia chena*, syntypes C.100582 (1), C.200849 (many), Chichester Dam, Hunter, NSW.

Material Dissected

Northern Territory – Mulura Rapids, Roper Ri. at Elsie Park, 14°56’40”S, 133°12’22”E, 27 Aug. 1994 (C.201826); *Queensland* Ban Ban Springs, off Burnett Hwy., E of Gayndah, 25°41’06”S, 151°48’54”E, 29 May 1992 (C.203019); *New South Wales* – Clarence Ri., on N bank, about 1 km upstream of bridge,



FIGS. 7–12. *Posticobia* species. FIGS. 7–10: *P. brazieri*; FIG. 7: North bank of Clarence River, Grafton, New South Wales (C.203018); FIG. 8: Mungo Brush, Myall Lakes, New South Wales (C.203021); FIG. 9: Murray River, Murray Bridge, South Australia (C.203022); FIG. 10: Roper River, Elsie Park, Northern Territory (C.203024); FIG. 11: ***P. ponderi*** Mulligan Springs, Lake Callabonna, South Australia (C.203023); FIG. 12: *P. norfolkensis* Norfolk Island, New South Wales (C.203025). Scale bars = 1 mm.

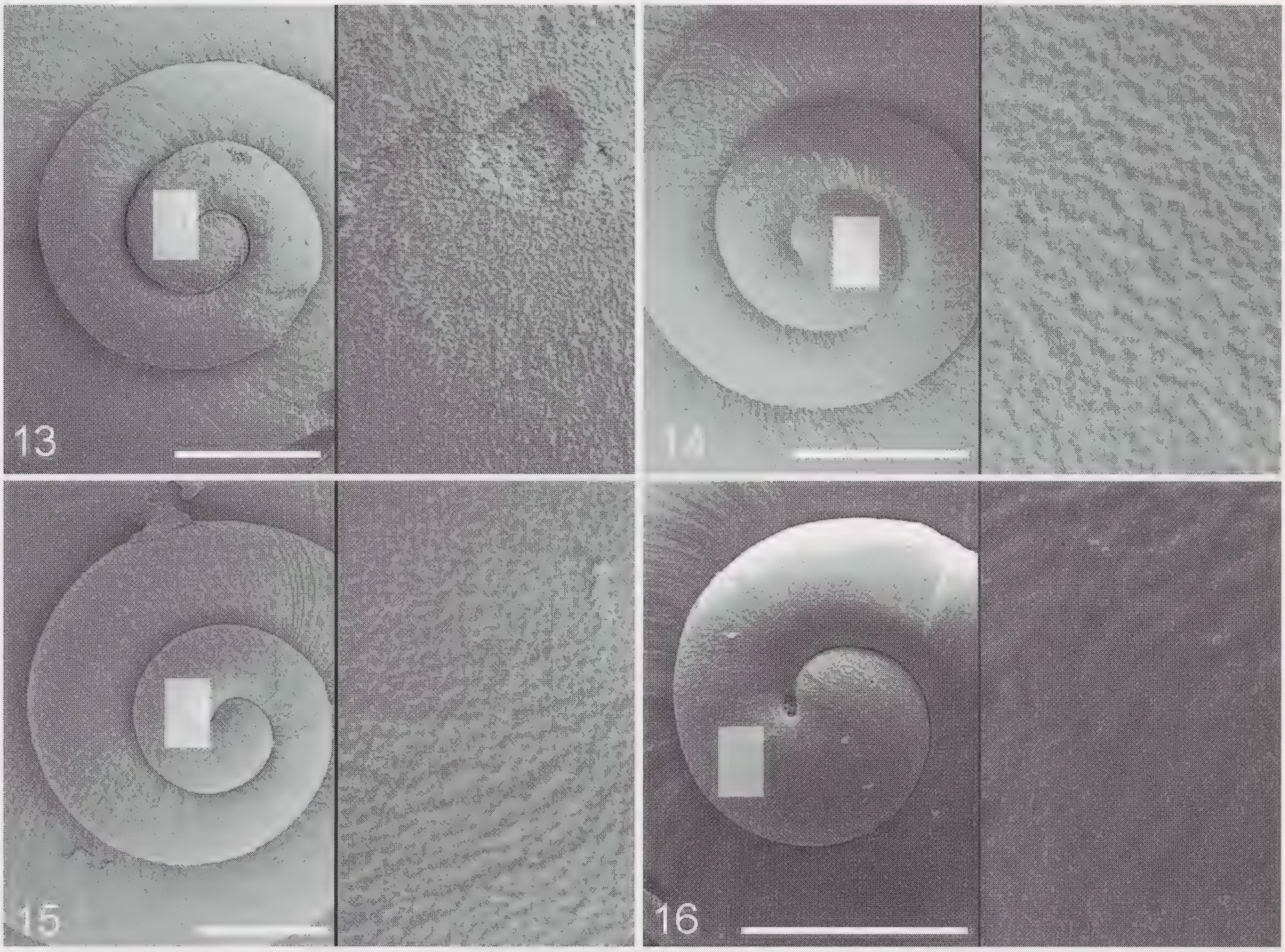
Grafton, 29°41’50”S, 152°56’05”E, 11 Mar. 1993 (C.203025); Mungo Brush, Myall Lakes NP., N of Hawks Nest, 32°32’33”S, 152°18’33”E, 10 Jan. 1993 (C.203028); Halfway Point, Lane Cove Ri., Lane Cove River NP., Chatswood West, Sydney, 33°47’29”S, 151°08’47”E, 13 Sept. 1992, S.4008 (C.203029); *South Australia* – Murra Ri. at Murray Bridge, 35°07’18”S, 139°16’53”E 30 May 1993 (C.203034).

Material Examined

Northern Territory – Roper Ri. at Roper Bar camping ground, 14°42’42”S, 134°30’46”E, 20 May 1997 (C.331884); Roper Ri. at Rocky Bar Crossing, Roper Valley Station, 14°44’13”S, 134°02’58”E, 22 Aug. 1995 (C.202314); 40 m above Elsie Falls, Roper Ri., 14°55’05”S, 133°07’04”E, 20 Aug. 1994 (C.201825); Elsie Falls at Roper Ri., 14°55’05”S, 133°07’03”E, 20 Aug. 1994 (C.410923); Mulurak Rapids, Roper Ri. at Elsie Park, 14°56’40”S, 133°12’22”E, 27 Aug. 1994 (C.201826, C.327897); *Queensland* – Tributary of Flaggy Cr., Little Mulgrave Ri., on Little Mulgrave Rd. ca. 4 km from Gillies Hwy., 17°07’23”S, 145°41’46”E, 28 Apr. 1998 (C.381712); Little Mulgrave Ri. at base of escarpment, 17°08’30”S, 145°44’23”E, 5 June 1997 (C.326804); Beames Brook, SW of Burketown, 17°52’43”S, 139°20'34"E, 28 May 1997 (C.346421); Running creek on Gregory Downs-Burketown Rd., 40 km N of Gregory Downs and about 5 km S of Almora Station, 18°19'24"S, 139°15'49"E, 28 May 1997 (C.410921); Burdekin Ri., 18°30'S", 145°19'58"E, 15 Nov. 1983 (C.142292); 2 km downstream from waterhole behind Lawn Hill Station homestead, 18°34'02"S, 138°35'08"E, 26 May 1997 (C.346420); Lawn Hill Cr. within 1 km of National Park ranger station, 18°42'S, 138°29'E, 26 May 1997 (C.410919); Gregory Ri. on Riversleigh-Lawn Hill Rd., 19°01'06"S, 138°43'33"E, 27 May 1997 (C.331855); Three Mile Cr., Palarenda, Townsville, 19°12'37"S, 146°46'30"E, 23 Feb. 1997 (C.331874); Myrtle Cr., crossing No 1 at Bruce Hwy., ca. 5 miles N of Proserpine, 20°21'S, 148°33'E, 4 May 1975 (C.200815); Pine Tree Cr. Lagoon, Hughenden, 20°51'S, 144°11'58"E, 12 June 1983 (C.200887); Keppel Bay, Yeppoon, 23°07'58"S, 150°45'E (C.200860); Double Cr., SW of Calliope, 24°02'54"S, 151°09'E, 8 May 1992 (C.203017); Boyne Ri. at Rosedale, S of Gladstone, 24°13'40"S, 151°15'28"E, 5 Sept. 1982 (C.200881); Skeleton Cr. at Bruce Hwy., N of Miriam Vale, 24°19'16"S, 151°32'18"E, 7 May 1992 (C.203018); Double Cr. at Dawson Hwy., SW of Gladstone, 24°45'S, 151°08'13"E, 5 Sept. 1982 (C.200810); Belinda Springs, Salvator Rosa Section, Carnarvon Gorge NP., 24°50'S, 147°11'45"E, 29 Sept. 1984 (C.156794, C.157168); Burnett Ri., 25°04'58"S, 152°00'E (C.011019, QM MO.26518); Burnett Ri. at Causeway Rd., N of Booyal, S of Gin Gin, 25°13'45"S, 152°42'E, Sept. 1982, July 1997 (C.200808, C.410915); Isis Ri. at Bruce Hwy., S of Childers, 25°16'08"S, 152°22'23"E, 9 May 1975 (C.200818); Burnett Ri. at Trurick Cr., W of Childers, 25°18'38"S, 151°56'15"E, 3 Sept. 1982 (C.200809); Eidsvold, 25°23'S, 151°08'E, 1912 (C.033769); Woowoonga Stream at Childers-Biggenden Rd., W of Childers, 25°28'S, 151°59'58"E, 4 Sept. 1970 (C.440028); Burnett Ri., E of Gayndah, 25°37'S, 151°36'E, 25 Apr. 1975 (C.200816); Eel Cr. at Biggenden-Maryborough Rd., ca. 20 km E of Biggenden, 25°37'S, 152°10'E, 8 July 1980 (QM MO.26529); Ban Ban Springs, off Burnett Hwy., E of Gayndah, 25°40'51"S, 151°48'56"E, 1982–2000 (C.200790, C.425869, C.203019, C.302414); Wide Bay Cr., NW of Gympie, 26°04'S, 152°14'48"E, 2 Sept. 1982 (C.200806, C.362225); Fat Hen Cr. at Wide Bay Hwy., 26°05'23"S, 152°17'15"E, 11 Sept. 2000 (C.386127); Widgie Cr. at Reids Rd., W of Gympie, 26°11'15"S, 152°28'45"E, 7 Sept. 1982 (C.200883); Eel Cr., SW of Gympie, 26°13'49"S, 152°37'52"E, 7 Sept. 1982 (C.200811); small stream 9.5 km from Myravale Forest Station, Glastonbury SF, W of Gympie, 26°14'49"S, 152°29'30"E, 7 Sept. 1982 (C.200880); tributary of Marys Cr., SW of Gympie, 26°16'01"S, 152°32'52"E, 7 Sept. 1982 (C.200812); Barambah Cr. at Central Burnett Hwy., E of Gayndah, 26°19'58"S, 152°11'58"E, Apr. 1975, Sept. 1982 (C.200819, C.200805); Skyring Cr. at Amama Picnic ground in Amamoora SF, 26°21'46"S, 152°38'04"E, 7 May 1998 (C.410918); Eumundi, Templeton Property, 26°29'25"S, 152°57'12"E, 30 Sept. 2003 (C.456847); Derrier Cr. at Imbil Forest Dr., 26°30'23"S, 152°37'56"E, 7 May 1998 (C.410920); Coonoon Cr. at Gibber Rd., in Brooloo SF, S of Gympie, 26°32'30"S, 152°41'30"E, 7 Sept. 1982 (C.200814); Old Coonon Gibber Cr., Brooloo SF, SW of Gympie, 26°34'45"S, 152°40'E, 7 Sept. 1982 (C.200813); Picnic Cr., above walking track at or near edge of Kondalilla NP, 26°40'18"S, 152°52'05"E, 6 May 1998 (C.401222); tributary of Little Yabba Cr., 4.4 km S of Jimna, 26°41'35"S, 152°24'21"E, 8 Mar. 1993 (C.203020); Neunum Cr., just off Somerset Dam, N of Brisbane, 27°01'08"S, 152°41'51"E, 30 Aug. 1982 (C.200826); North Pine Ri., 1 km S of Mt. Pleasant, SW of Caboolture, 27°09'45"S, 152°46'28"E, 30 Aug. 1982 (C.200882); Dayboro, Brisbane, 27°10'S, 152°50'E, 11 Aug. 1976 (QM); tributary of Laceys Cr. at Laceys Creek Rd., 27°11'45"S, 152°45'15"E, 30 Aug. 1982 (C.200825); tributary of North Pine Ri., Dayboro, NW of Petrie, 27°13'04"S, 152°49'58"E, 10 May 1975 (C.200840); Cedar Cr. at Dayboro-Samford Rd., 27°13'58"S, 152°43'58"E, 10 May 1975 (C.200843); Rush Cr. at Dayboro Rd., 10 km NW of Petrie, 27°13'58"S, 152°53'58"E, 20 Jan. 1981 (C.200885); North Pine Ri., Dayboro, Brisbane, 27°15'S, 152°50'E, 11 Aug. 1976 (QM); Lower Pine Ri., 27°16'58"S 153°01'E (C.200862); North Pine Ri., Brisbane, 27°16'S, 152°58'58"E, 1912, Aug. 1979 (C.200859, C.200861, C.033713); North Pine Ri. at Dayboro-Samford Rd., 2 miles S of Dayboro, 27°19'58"S, 152°52'E, 10 May 1975 (C.200841); North Pine Ri. at Stafford Rd., LHS of bridge, 2 km W of Dayboro, 27°19'S, 152°52'58"E, 20 Jan. 1981 (C.200886); Cedar Cr., Closeburn, Brisbane, 27°20'S, 152°50'E, 11 Aug. 1976 (QM); South Pine Ri. at Dayboro-Samford Rd., 1 mi N of Samford, 27°22'S, 152°52'58"E, 10 May 1975 (C.200842); Kedran Brook, Brook St., Brisbane, 27°23'58"S, 152°58'E, 1 Aug.

1976 (C.200838, QM); Brisbane Ri., McFarlanes Bridge, near Bryden, between Northbrook & Esk, 27°23’S, 152°37’E, 20 Jan. 1981 (QM); Dawson Cr. at “Goat Track”, near Mt. Nebo, W of Brisbane, 27°24’S, 152°47’50”E, 9 Mar. 1993 (C.203021); Enoggera Cr. at Ashgrove, Brisbane, 27°28’S, 153°00’E, 27 Dec. 1981 (C.200867); Indooroopilly, Brisbane, 27°28’S, 153°02’E (QM MO.26525); Kenmore, Brisbane, 27°30’S, 152°50’E, 15 Aug. 1976 (QM); Moggill Cr., Brookfield, 27°30’S, 152°56’E, Aug. 1976 (QM); Mt. Crosby, Brisbane, 27°30’S, 152°45’E, 15 Aug. 1976 (QM); Moggill Cr., Brisbane, 27°31’58”S, 152°55’58”E, July 1958, Feb. 1984 (C.200837, C.142291); Moggill Cr., Brookfield, Brisbane, 27°32’S, 152°56’E, 31 Oct. 1949 (QM MO.26517); Moggill & Gold Crs., Brookfield, Brisbane, 27°32’S, 152°56’E, 8 Apr. 1951 (QM MO.26519); Nerang Ri., Latimers Crossing, S of Nerang, 28°01’33”S, 153°17’51”E, 30 May 1992 (C.203022); in gully beside picnic area, Numinbah Valley SF, 28°07’33”S, 153°13’45”E, 17 Dec. 1980 (QM MO.26520); Waterfall Cr., junction of Springbrook Rd. and road to Numinbah Valley, 28°06’29”S, 153°14’38”E, 18 Mar. 1981 (C.128694); New Year (Tartars) Cr., 3 km from junction with Levers Plateau Rd., ENE of Woodenbong, 28°17’25”S, 152°50’50”E, 17 Mar. 1981 (C.200830); *New South Wales* – S end of Cudgen Lake, 28°19’39”S, 153°33’28”E, 27 Mar. 1998 (C.339281); Hoffmans Cr. at Woodenbong-Wylie Creek Rd., 28°25’58”S, 152°23’58”E, 9 Sept. 1982 (C.200828); tributary of Hoffmans Cr., Hewetsons Hill, ca. 14 km E of Legume, 28°25’59”S, 152°23’32”E, 15 Mar. 1981 (C.128692); Burringbar Cr. at Pacific Hwy., 48 km S of Tweed Heads, 28°26’19”S, 153°28’30”E, 18 Oct. 1976 (C.200836); Oakey Cr., W of Legume, 28°26’30”S, 152°22’58”E, 9 Sept. 1982 (C.200827); ford at Byrrill Creek Rd., Pretty Gully, N of Nimbin, 28°26’36”S, 153°14’30”E, 19 Mar. 1981 (C.200831); tributary of Byrrill Creek at Mebbin Forest Rd., 1 km from Byrrill Creek Rd. junction, Mebbin SF, 28°26’38”S, 153°11’48”E, 20 Mar. 1981 (C.128696); Hoffmans Cr., 800 m N of Tooloom turnoff at Hewetsons Mill at Mt. Lindsay Hwy., SE of Warwick, 28°26’S, 152°23’36”E, 15 Mar. 1981 (C.200768); Richmond Ri. at Kyogle, 28°36’52”S, 152°59’21”E, 9 May 1997 (C.428867); Taylors Lake, via Byron Bay, 28°37’30”S, 153°36’E, 22 July 1976 (C.200835); Horse Station Cr. at Omagh Rd., SW of Kyogle, 28°38’57”S, 152°58’40”E, 12 Aug. 1991 (C.167445); tributary of Peacock Creek at Peacock Creek Rd., 2.3 km S of Sandy Cr. Forest Rd., NE of Bonalbo, 28°41’11”S, 152°41’49”E, 14 Mar. 1981 (C.200829); tributary of Richmond Ri. at Edenville Rd., NW of Casino, 28°43’54”S, 152°57’59”E, 12 Aug. 1991 (C.167448); Booerie Cr. at Bentley Rd., N of Lismore, 28°45’58”S, 153°17’05”E, 11 Mar. 1993 (C.203023); Lagoon Cr. at Summerland Way, Fairy Hill, N of Casino, 28°46’08”S, 152°59’06”E, 12 Aug. 1991 (C.167655); Wilson Ri. at Banglow Rd., 7 km NE of Lismore, NSW 28°46’49”S, 153°19’54”E, 11 Mar. 1993 S.5072 (C.203024); in small stream, below foot bridge, Rotary Park, Lismore, 28°48’42”S, 153°17’43”E, Oct.–Dec. 1993 (C.201405, C.201404); in a small stream, Wilsons Nature Reserve, Lismore, 28°50’04”S, 153°17’11”E, 16 Dec. 1993 (C.200899); Wyrallah Rd., Wilson Nature Reserve, Lismore, 28°50’S, 153°17’30”E, 15 Dec. 1998 (C.364918); Richmond Ri., Casino, 28°52’S, 153°34’E, 1970–1982 (C.200869, C.200824, C.200823); Richmond Ri., near & above Lismore, 28°59’S, 153°16’E, 1882 (C.019919); Richmond Ri., Woodburn, 29°04’30”S, 153°20’30”E, 17 Oct. 1976 (C.200832); Tuckombil Canal at Pacific Hwy., S of Woodburn, 29°05’S, 153°20’18”E, 26 Mar. 1998 (C.338933); Clarence Ri. at Baryulgil crossing, S of Tabulum, 29°13’01”S, 152°33’42”E, 28 Aug. 1982 (C.200821); Washpool Cr., 5.3 km S along Washpool Rd. from Lionsville Rd. turnoff, Washpool SF, SW of Casino, 29°13’37”S, 152°28’56”E, 29 Aug. 1982 (C.200822); Clarence Ri., 0.8 km E of Maclean, 29°27’S, 153°12’28”E, 18 Oct. 1972 (C.200834); Blue Pools near Angourie, 5 km S of Yamba, 29°28’45”S, 153°21’48”E, Sept. 1982–1984 (C.200889, C.200839); Ingalbar Cr. at Jackadgery Rd., NW of Grafton, 29°34’13”S, 152°33’46”E, 27 Aug. 1982 (C.200820); Clarence Ri. at Jackadgery Rd., 1 km W of Lilydale, 40 km W of Grafton, 29°34’58”S, 152°33’E, 4 Feb. 1981 (C.200884); Coldstream Ri. at Tucabia, 29°40’02”S, 153°06’16”E, 26 Mar. 1998 (C.338935); in swamp, Clarence Ri., South Grafton, 29°40’S, 152°55’58”E, 1900 (C.200865); Clarence Ri. at South Grafton, 29°40’S, 152°55’58”E, 1877 (C.200866); Clarence Ri., ca. 1 km upstream of bridge, Grafton, 29°41’49”S, 152°35’07”E, 11 Mar. 1993 (C.203018); Clarence Ri., South Grafton, 29°42’02”S, 152°56’26”E, 1870, 1912 (C.55145, C.200863); stream running into Clarence Ri., Grafton, 29°42’S, 152°55’58”E, 1914 (C.200864); Nymboida Ri., N bank on Old Glen Innes Rd., near Buccarumbi, NW of Nymboida, 29°50’13”S, 152°35’07”E, 12 Mar. 1993 (C.203026); Corindi Ri., 30°01’19”S, 153°11’31”E, 26 Mar. 1998 (C.338934); Namoi

Ri. at Tarriaro Bridge, on Narrabri-Turrawan Rd., 30°22'S, 149°51'28"E, 14 June 1975 (C.200833); Sandy Cr., S of Narrabri, 30°23'46"S, 149°48'57"E, 1887 (C.391747); Namoi Ri. near Gunnedah, 30°58'23"S, 150°15'16"E, 1967 (C.200868); Peel Ri., Bective near Tamworth, 30°58'58"S, 150°43'58"E, 1880's (C.309885); Kendall near Wauchope, 31°37'58"S, 152°42'28"E (C.200850); Manning Ri., Wingham Brush, W of Taree, 31°52'15"S, 152°22'51"E, 26 Aug. 1982 (C.200844); stream, 3 km S of Coolongolook Ri. crossing on edge of Pacific Hwy., S of Taree, 32°15'29"S, 152°19'25"E, 26 Aug. 1982 (C.200888); Muscle Cr. at Bell St. bridge crossing, Muswellbrook, 32°16'15"S, 150°52'39"E, 24 May, 27 June 2003 (C.456333, C.456331); Muscle Cr. at New England Hwy., between pedestrian overpass bridges at collapsed weir, ca. 2 km E of Muswellbrook, 32°16'47"S, 150°54'19"E, 24 May 2003 (C.456340); Muscle Cr. at New England Hwy., behind Pinaroo Caravan Park, ca. 2 km E of Muswellbrook, 32°16'53"S, 150°54'27"E, 14 May 2001 (C.456346); Muscle Cr. at Muswellbrook, 32°16'S, 150°54'E, 18 Sept. 1996 (C.360245); Williams Ri. between railway bridge and road bridge beside park, Dungog, 32°23'47"S, 151°45'45"E, 15 Feb. 2003 (C.421945, C.456342); Myall Lakes, 2 km W of Smith Lake, 32°23'S, 152°26'E, 16 Jan. 1964 (C.200848); Fal Brook at Goorangoola Rd., Mt. Pleasant, N of Singleton, 32°24'37"S 151°11'36"E, 30 May 1903, 1 Dec 1999 (C.456344, C.456337); Myall Ri. at Bulahdelah, 32°24'54"S, 152°12'21"E, 1 Nov. 1992 (C.203027); Bulahdelah, 32°25'S, 152°12'E, 1925, 1994 (C.200870, C.200871); Bombah Point, near ferry, Bombah Broadwater, Myall Lakes NP, 32°30'20"S, 152°18'17"E, 13 Feb. 1989 (C.200851); Wattle Ponds at Bridgemans Rd., Hunterview, N of Singleton, 32°32'27"S, 151°10'06"E, 24 Sept. 2001 (C.456339); Blackfellows Bay, Mungo Brush, Myall Lakes NP, 32°32'36"S, 152°18'21"E, 13 Feb. 1989 (C.300610); Singleton, 32°34'S, 151°10'E, 1924 (C.51765); along Stony Cr. at edge of Uffington SF, behind Clarence Town Cemetery, off Cemetery Rd., NW of Newcastle, 32°35'13"S, 151°45'37"E, 10 Jan. 2002 (C.456349); creek at Nelson Rd., under bridge beside railway station, Greta, 32°41'12"S, 151°23'04"E, 19 June 2001 (C.456334); Black Cr. at Old North Rd., Rothbury, NW of Newcastle, 32°42'45"S, 151°19'25"E, 20 June 2001 (C.456347); nature reserve next to oval, University of Newcastle, Callaghan, 32°53'40"S, 151°42'05"E, Aug. 1999, Sept. 2000 (C.456343, C.456348); tributary of Wallis Cr., ca 500 m upstream from Wallis Cr. junction, N of Sandy Creek Rd. crossing, Bow Wow Gorge, 5 km SW of Mulbring, 32°55'37"S, 151°26'24"E, 20 Sept. 2003 (C.456335); tributary of Wallis Cr., N of Sandy Creek Road crossing, Bow Wow Gorge, 5 km SW of Mulbring, 32°55'40"S, 151°26'23"E, 20 Sept. 2003 (C.456336); behind inlet weir of small creek beside scout hall, Kotara Park, Kotara, 32°56'34"S, 151°41'46"E, 2 Aug. 2003 (C.456338); Flaggy Cr. at track above waterfall, Glenrock State Recreational Area, Highfields, 32°57'36"S, 151°43'46"E, 11 Aug. 1999 (C.456341); Congewai Cr. at Congewai Rd., Congewai, 32°57'53"S, 151°16'10"E, 8 Mar. 2003 (C.456332); S end of Dudley Beach, Glenrock State Recreational Area, Dudley, 32°58'55"S, 151°43'37"E, 26 Aug. 1999 (C.456345); small creek behind Redhead Beach Caravan Park, S of Newcastle, 33°01'S, 151°42'28"E, 25 Dec. 1991 (C.200872); Dora Cr., Cooranbong, 33°04'58"S, 151°26'58"E, 5 Apr. 1975 (C.200845); Cattai Cr. between crossings of Cattai Ridge & Newman Rds., Maraylya, 33°34'57"S, 150°56'22"E, 5 Aug. 1994 (C.320653); Cattai Cr. at Cattai Ridge Rd., Maraylya, 33°35'01"S, 150°56'23"E, 3 Oct. 1994 (C.321254); Cattai Cr., upstream side of McClymonts Rd., Maraylya, 33°37'01"S, 150°55'39"E, 3 Oct. 1994 (C.321262); South Cr., upstream of Blighs Park/South Windsor STP outfall, Blighs Park, 33°38'30"S, 150°49'19"E, 24 July 1995 (C.309218); Lane Cove Ri., Lane Cove River State Recreation Area, Halfway Point picnic area, Chatswood West, Sydney, 33°47'16"S, 151°08'55"E, Feb. 1993, Oct. 1994 (C.320244, C.321280); Cabramatta Cr., between Hume Hwy. & railway line, Liverpool, Sydney, 33°54'17"S, 150°56'30"E, Aug. 1973, Nov. 1991 (C.200846, C.320700); Cabramatta Cr., Mt. Pritchard, Sydney, NSW, 33°54'18.5"S, 150°55'15"E, 8 Aug. 1992 (C.203030); Cabramatta Cr., Irelands Bridge Reserve, W of Hume Hwy., Warwick Farm, Sydney, 33°54'17.5"S, 150°56'37"E, 18 June 1989, 11 Oct. 1992 (C.200847, C.203031); Georges Ri. at Newbridge Rd., Liverpool, 33°55'46"S, 150°55'41"E, 13 Nov. 1994 (C.320661, C.321551); Georges Ri. at Cambridge Ave. causeway, Glenfield, 33°58'22"S, 150°54'38"E, 8 Jan. 1995 (C.320714); *Victoria* Wimmera Ri., 1 km NW of Jeparit, 36°09'S, 141°58'59"E, 10 Sept. 1977 (NMV F.54797); Wimmera Ri., Jeparit, 36°09'S, 141°58'59"E, 26 May 1978 (NMV F.54801); Wimmera Ri., 2 mi W of Dimboola, 36°27'S,



FIGS. 13–16. Protoconchs of *Posticobia* species. FIGS. 13, 14: *P. brazieri*; FIG. 13: North bank of Clarence River, Grafton, New South Wales; FIG. 14: Mungo Brush, Myall Lakes, New South Wales; FIG. 15: ***P. ponderi*** Mulligan Springs, Lake Callabonna, South Australia; FIG. 16: *P. norfolkensis* Norfolk Island, New South Wales. Right hand portion of figures shows detail of protoconch microsculpture (x8). Scale bar = 200 µm.

142°02'E, Apr. 1971 – Apr. 1972 (C.200853, NMV, NMV F.54743); Wimmera Ri., downstream of Dimboola Ri., 36°27'S, 142°00'E, 5 May 1986 (C.200856); Wimmera Ri., upstream, Horsham, 36°42'S, 142°13'E, 10 Nov. 1987 (C.200854); Wimmera Ri., upstream from the McKenzie Ri., S of Horsham, 36°44'S, 142°10'E, Nov. 1986–1987 (C.201416, C.200855); Glenelg Ri., 37°09'S, 141°51'E, 3 June 1994 (C.203998); *South Australia* Port Pirie, 33°11'S, 138°01'E 1905, 1958 (SAM D.55, D.11931); River Broughton, Koolunga, 33°35'S, 138°20'E (SAM D.11956); Morgan-Whyalla pipeline, 34°02'S, 139°40'E, 22 Aug. 1958 (SAM D.65); Wakefield Ri., 10 miles W of Riverton, 34°08'S, 138°28'E (SAM D.11939); Wakefield Ri. at Robins Crossing, E of Balaklava, 34°09'41"S, 138°34'06"E, 10 June 1994 (C.203033); Light Ri. at Mirgays Waterhole, 34°20'S, 139°00'E, 25 May 1999 (C.405557); Torrens Ri. near Tepleys Hill Rd. Bridge, Fulham, Adelaide, 34°55'54"S, 138°31'02"E, 28 Apr. 1980 (C.200858); Murray Ri., Murray Bridge, 35°07'18"S, 139°16'53"E, Sept. 1981 (C.200857); Rodwell Cr. tributary of Bremer Ri., Woodchester, 35°12'S, 138°57'E, 25 Apr. 1994 (C.410922); Murray Ri. at Tailem Bend, 35°15'30"S, 139°27'08"E (SAM); Angas Ri., Strathalbyn, 35°16'S, 138°54'E (SAM); Lake Alexandrina, 35°25'S, 139°20'E (SAM D.56); Point McLeay, Lake Alexandrina, eastern side, 35°31'01"S, 139°07'59"E (SAM D.11937).

Diagnosis

Shell 1.37–3.31 mm in height, 1.18–2.82 mm in width, broadly conical; sutures slightly to moderately impressed; body whorl with weak to well developed keel; umbilicus partially open to narrow. Operculum yellow, with 3–5 weakly developed pegs on inner side.

TABLE 1. Shell measurements for *Posticobia* species. Note: operculum either missing or broken in sample of *P. norfolkensis* shells measured.

	SH	SW	AH	BW	CV	TW	OL	OW	OSL	OSW
P. brazieri (n = 337)										
Minimum	1.37	1.18	0.68	1.08	0.10	2.25	0.65	0.51	0.06	0.03
Maximum	3.31	2.82	1.52	2.42	0.32	4.30	1.47	1.06	0.57	0.40
Median	2.47	1.98	1.17	1.87	0.19	3.30	1.04	0.81	0.32	0.18
Mean	2.45	1.97	1.17	1.85	0.19	3.32	1.04	0.81	0.33	0.19
SD	0.35	0.26	0.16	0.24	0.04	0.32	0.14	0.10	0.08	0.06
P. ponderi (n = 30)										
Minimum	1.68	1.41	0.82	1.27	0.14	2.55	0.77	0.64	0.17	0.07
Maximum	2.60	1.98	1.20	1.85	0.28	3.75	1.08	0.90	0.35	0.19
Median	2.22	1.69	1.03	1.66	0.21	3.20	0.94	0.75	0.26	0.12
Mean	2.19	1.71	1.04	1.63	0.21	3.17	0.93	0.74	0.26	0.12
SD	0.18	0.12	0.09	0.12	0.03	0.24	0.07	0.06	0.05	0.03
P. norfolkensis (n = 20)										
Minimum	2.11	1.91	1.07	1.78	0.18	2.65				
Maximum	2.71	2.35	1.37	2.15	0.35	3.50				
Median	2.41	2.06	1.26	1.92	0.25	3.05				
Mean	2.39	2.09	1.26	1.93	0.24	3.07				
SD	0.15	0.11	0.07	0.09	0.04	0.21				

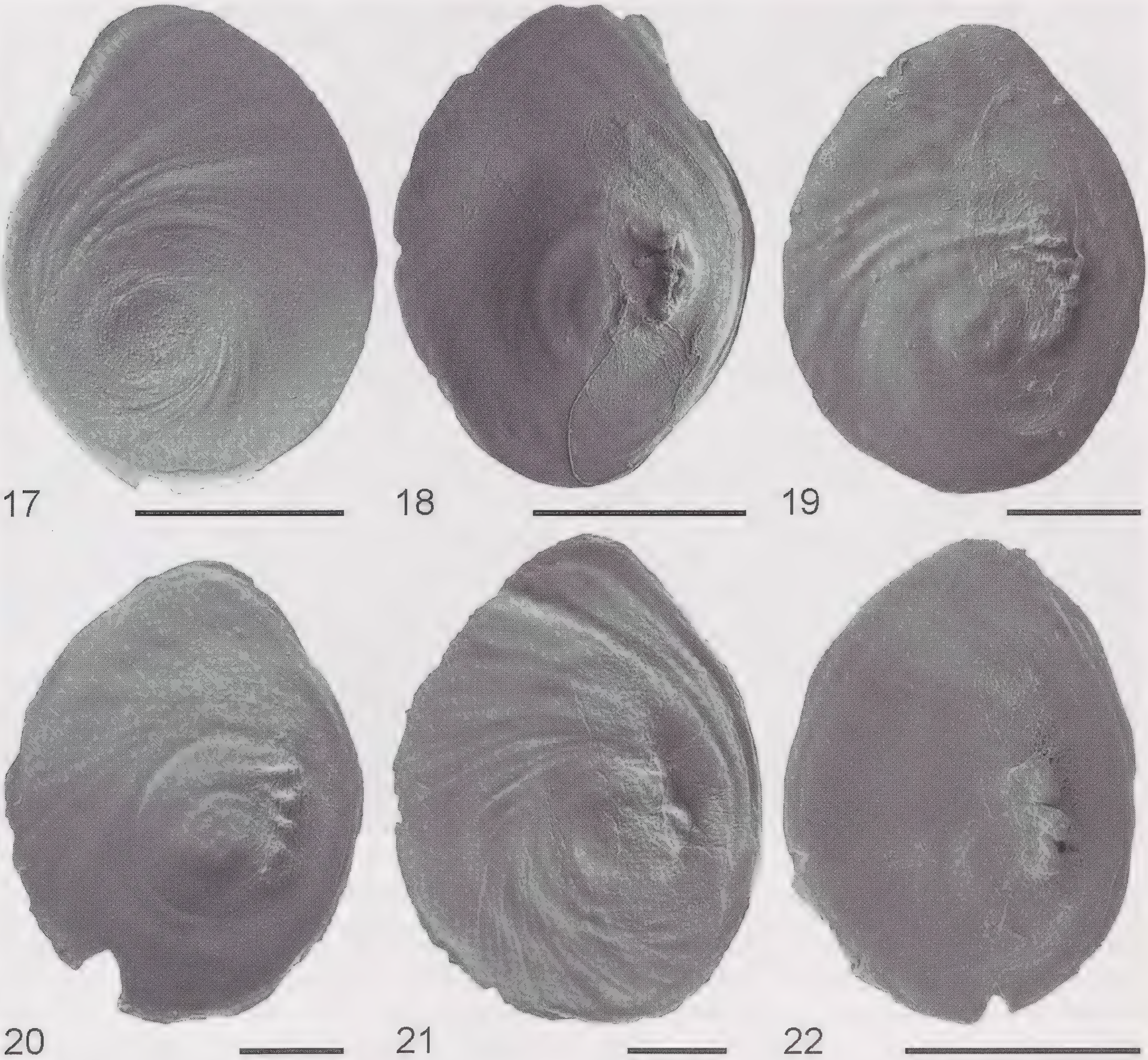
Description

Shell (Figs. 2–4, 7–10) – Shell 1.37–3.31 mm in height, 1.18–2.82 mm in width, broadly conical in shape; spire of average height, outline convex; sutures slightly to moderately impressed. Aperture 0.68–1.52 mm in height, oval and not disjunct from the last whorl. Teleoconch of 2.25–4.30 convex whorls; body whorl 1.08–2.42 mm in height; last whorl convex, weakly to strongly keeled. Teleoconch sculpture smooth, with fine to coarse growth lines. Inner lip of aperture firmly adhering to parietal wall. Outer lip weakly to heavily thickened, lacking an external varix, not reflected. Umbilicus partially open to narrow in adults, narrowly open in juveniles. Colour opaque, yellowish-brown, periostracum thin and may impart darker colour. Protoconch of about 0.85–1.30 whorls; sculptured with dense, irregular shallow pits. See Table 1 for the mean, median and standard deviation of shell dimensions.

Operculum (Figs. 17–20) – Ovate, thin, flat, yellow, horny, paucispiral, with eccentric nucleus, 0.65–1.47 mm in length and 0.51–1.06 mm in width. White smear 0.06–0.57 mm in length, 0.03–0.40 mm in width, 3–5 weakly developed pegs on inner side. Table 1 provides mean, median and standard deviation of opercular dimensions.

Radula (Figs. 23, 24) – Central teeth with 4–5 lateral cusps, sharply pointed and about 2 times longer than adjacent cusps; 3–4 pairs of basal cusps; basal tongue U-shaped, just protruding past lateral edge. Lateral teeth: with 4–5 cusps on each side, sharply pointed and about 2 times longer than adjacent cusps; basal projection U-shaped, lateral flange longer than cutting edge. Inner marginal teeth with 21–31 cusps. Outer marginal teeth with 25–35 cusps.

External Features – Animal pigmentation variable, from weakly to well developed (grey to black), cephalic tentacles long, slender, slightly tapering, with narrow, median-dorsal unpigmented line (corresponding to line of dorsal cilia). Snout (Fig. 27) with some cilia dorsally, with narrow, well-developed band laterally and latero-dorsally just behind labia. Tentacles with well-developed, mid-dorsal (Fig. 28) and two ventro-lateral (Fig. 29) strips of cilia.

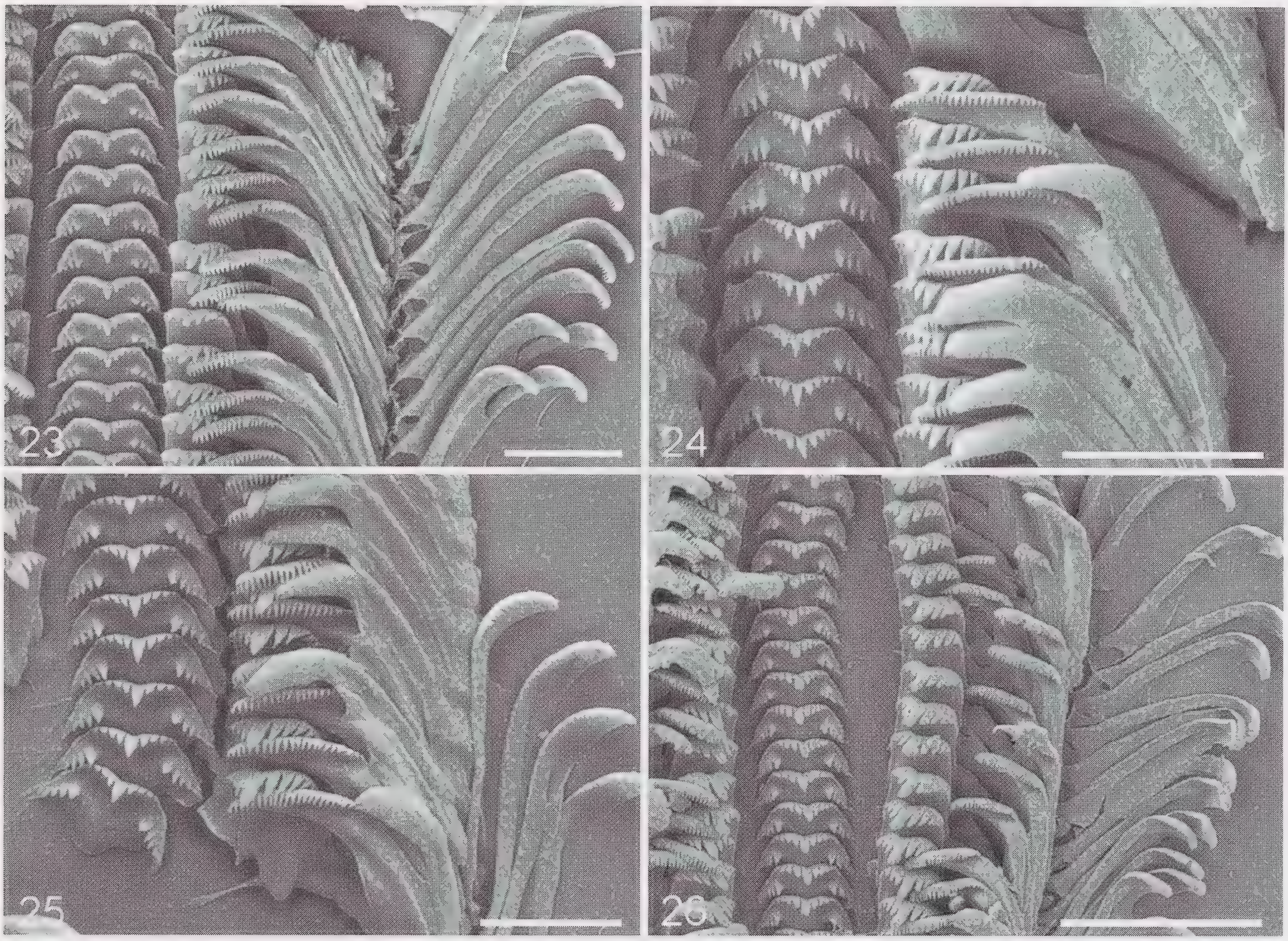


FIGS. 17–22. Opercula of *Posticobia* species. FIGS. 17–20: *P. brazieri*; FIGS. 17, 18: North bank of Clarence River, Grafton, New South Wales; FIG. 17: Outer side; FIG. 18: Inner side; FIG. 19: Mungo Brush, Myall Lakes, New South Wales; FIG. 20: Roper River, Elsie Park, Northern Territory; FIG. 21: ***P. ponderi*** Mulligan Springs, Lake Callabonna, South Australia; FIG. 22: *P. norfolkensis* Norfolk Island, New South Wales. Scale bars A, B, D, I = 0.5 mm; C, E–H = 0.2 mm.

Mantle Cavity – Ctenidium with 20–32 triangular filaments, width at widest point 0.12–0.39 mm, apices near right side, posterior end abutting pericardium. Osphradium 0.25–0.66 mm in length, white, elongately oval, located between posterior end and middle of ctenidium. Hypobranchial gland inconspicuous to well developed, partly to completely covering rectum. Rectum straight or with weakly developed arch (arch more developed in males). Visceral coil: Renal organ extends forward ca. 1/3–1/2 into pallial cavity, orientated longitudinally. Renal gland 0.34–0.72 mm in length, 0.16–0.34 mm in width. Pericardium more than 1/2 in pallial roof.

Stomach (Fig. 31) – Stomach 0.33–0.74 mm in length; style sac 0.22–0.67 mm in length, with very small to small gastric caecum 0.02–0.17 mm in length.

Male Genital System (Figs. 30, 32, 33) – Testis occupying about 1.0–1.5 whorls. Seminal vesicle beneath anterior 1/4 to 1/2 of first whorl of testis behind stomach; loosely to tightly coiled over stomach; conspicuously coiled on both digestive gland and testis behind stomach. Prostate gland (Fig. 32) 0.40–0.73 mm in length, 0.19–0.34 mm in width, about 1/3 to 2/3 in pallial roof, kidney-shaped, oval



FIGS. 23–26. Radulae of *Posticobia* species. FIGS. 23, 24: *P. brazieri*; FIG. 23: North bank of Clarence River, Grafton, New South Wales; FIG. 24: Mungo Brush, Myall Lakes, New South Wales; FIG. 25: ***P. ponderi*** Mulligan Springs, Lake Callabonna, South Australia; FIG. 26: *P. norfolkensis* Norfolk Island, New South Wales. Scale bars A, C, F = 50 µm; B, D, E = 20 µm.

to compressed-oval in section. Pallial vas deferens flush with surface of neck, slightly to strongly undulating from prostate gland to base of penis. Penis (Figs. 30, 33) unpigmented, distal part short, tapering, base broad, creased (when at rest). Penial duct strongly undulating in base and straight in distal portion of penis. Penis located just to right of midline, 0.28–0.56 mm behind right eye.

Female Genital System (Figs. 34, 35) – Ovary occupying about 1.00–1.40 whorls. Coiled oviduct with initial U-shaped bend orientated obliquely backwards; initial loop high to medium; proximal part simple; distal part straight or with one bend distal to seminal receptacle. Coiled oviduct and bursal duct joining just behind pallial wall, with oviduct joining bursal duct from right side. Oviduct straight, before joining with bursal duct. Bursa copulatrix 0.27–0.55 mm in length, 0.17–0.33 mm in width; bursal duct 0.04–0.12 mm in length, 0.07–0.15 mm in width, parallel sided, straight. Seminal receptacle located on or near anterior edge of bursa copulatrix to towards middle of inner wall of bursa copulatrix. Seminal receptacle 0.09–0.22 mm in length, 0.06–0.13 mm in width, pyriform in shape to a narrow sac with short duct. Rectum overlapping both albumen and capsule glands. Albumen gland 0.31–0.51 mm in length, 0.22–0.43 mm in width, and about 1/2 to 2/3 of albumen gland in front of posterior pallial wall. Capsule gland 0.34–0.90 mm in length, 0.18–0.35 mm in width, with anterior end tapering to blunt, oval to compressed oval in section, with distinct glandular zones. Ventral channel indistinct to distinct, with indistinct to moderate sized muscular vestibule. Genital opening small to medium in length, overlapping anterior end of capsule gland.

Egg Capsules – Egg capsules small, 0.30–0.40 mm in diameter, hemispherical in shape, contain a single egg and usually a greenish-yellow colour. They are found attached to various substrata, such as sand grains and vegetation or, rarely, attached to the shell of other individuals or other molluscs. Egg capsules have been found in most samples collected during the length of this project. From observations based on a small colony maintained for most of 1993, it appears that *P. brazieri* breeds year around, with a spring/summer peak. The rate of egg production is unknown. However, based on observations of a single captive female, it could be one egg capsule per day. In contrast, Oswald et al. (1991) found that the European spring-dwelling hydrobiid *Bythinella dunkeri* (Frauenfeld, 1857) produced about one egg capsule a week. Longevity is unknown. However, based on laboratory observations, it is at least a year.

Distribution

Found mainly along the coastal areas of New South Wales and Queensland, with scattered populations in western Victoria, South Australia, and the Northern Territory (Fig. 1). There is a large gap in distribution from Sydney, New South Wales, south through to far western Victoria. A large number of sites have been sampled throughout the area over many years, revealing numerous described and undescribed species of *Austropyrgus* and *Victodrobia* Ponder & Clark, 2003, only. The gap between the Northern Territory sites and the Queensland sites is, however, relatively poorly sampled, and further sampling in the region should reveal additional populations of *Posticobia*.

Remarks

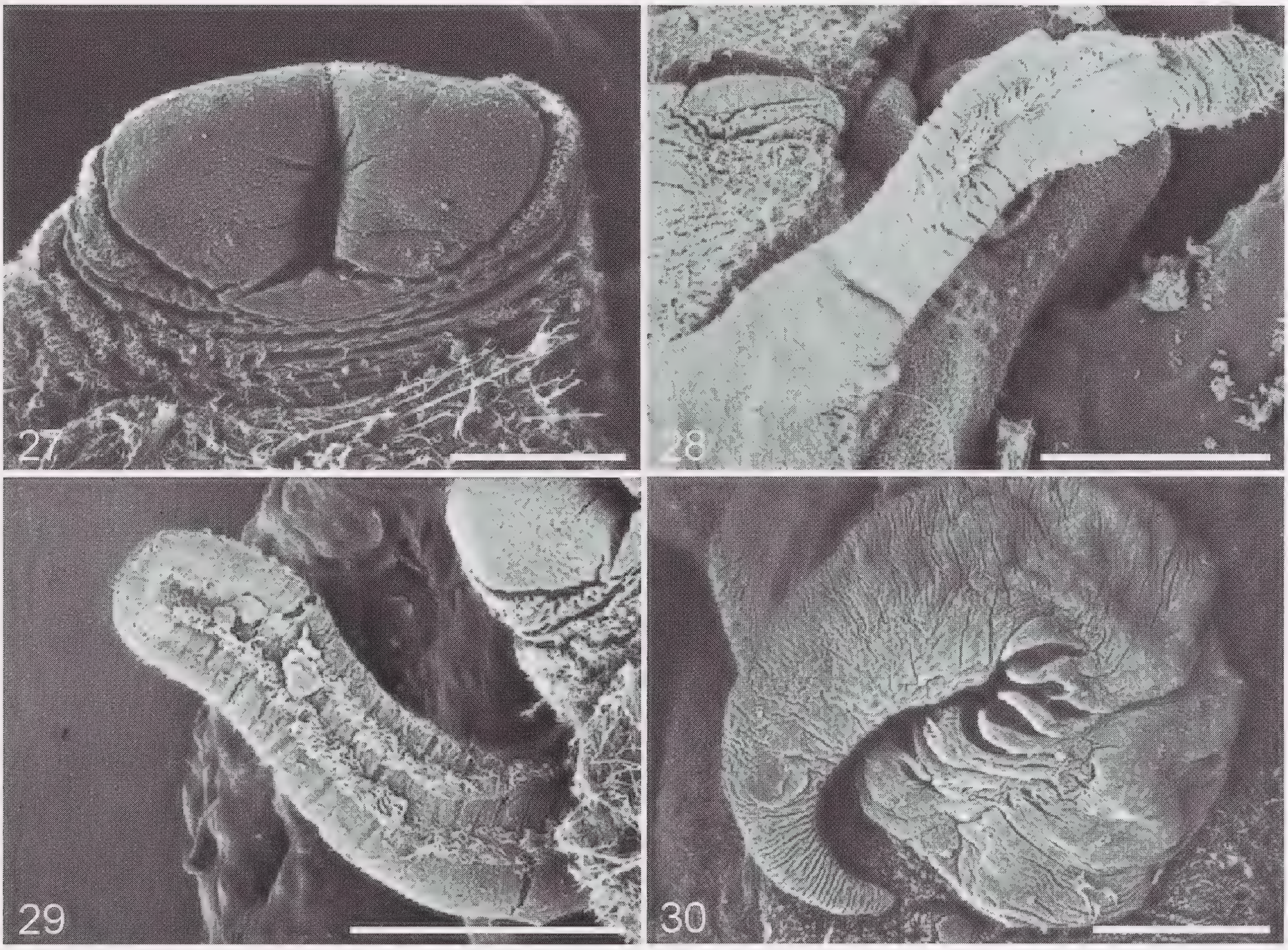
The shell morphology of *Amnicola positura* is very similar to that of *P. brazieri*, and Brazier (1889) was the first to recognise this and has been followed in this study. Smith (1992) was the first to synonymise *P. chena* with *P. brazier*, and this is supported by this study. According to notes with the original sample of *P. chena*, it came from the stomach contents of a duck shot on Chichester Dam. However, no specimens of *Posticobia* have ever been found at the dam, and the other molluscan taxa present in the sample – *Tatea rufilabris* (Adams, 1862), *Ascorhis tasmanica* (Martens, 1858) and *Fluviolanatus subtorta* (Dunker, 1857) – are all common, widespread species found in brackish conditions. These taxa and a morphologically similar form of *Posticobia* are representative of the fauna and conditions found nearby at the Myall Lakes. These snails are smaller, than typical *P. brazieri* found to the north, south and west of the lakes, including at the Myall River at Bulahdelah, some 17 km by river west of the lakes. Besides the size difference the other major difference is the degree of keel development, which varies from very weak to obvious.

Posticobia brazieri can be easily distinguished from all other known Australian hydrobiids by its shell shape and the presence of a keel in almost all populations. When *P. brazieri* is found in freshwater habitats, it is usually the only hydrobiid present. However, it is known to be sympatric with *Fluvidona petterdi* (Smith, 1882) at Lismore, northern New South Wales, and at least four undescribed species of *Fluvidona* and *Jardinella* in northern New South Wales and southern Queensland. It occurs with *Austropyrgus macropus* Clark et al., 2003, in a few localities in southeastern South Australia (Fig. 1) and with the introduced hydrobiid *Potamopyrgus antipodarum* (Gray, 1843) in several sites in Sydney and Newcastle, New South Wales, and southeastern South Australia. In estuarine conditions, such as the Myall Lakes, central New South Wales, it can be found with the estuarine hydrobiids *Ascorhis tasmanica* and *Tatea rufilabris*.

Discriminant function analysis using only shell measurements (SH, SW, AH, BW, CV, TW) separated all three taxa (Wilks' lambda = 0.632; approx. F = 19.619, df = 10,760 p-tail < 0.000), with 227 of 337 specimens of *P. brazieri* correctly identified, while 23 were misidentified as *P. norfolkensis* and 87 as ***P. ponderi*** n. sp. for an overall classification rate of 67%.

Given the range of shell variation observed in *P. brazieri* as currently recognised, it is possible that future molecular and anatomical studies may reveal additional taxa. However, Haase (2003) observed that the shell shape of populations of *Potamopyrgus antipodarum* from two streams on the North Island of New Zealand was correlated with flow, with shell size increasing downstream. A similar phenomenon seems to be occurring in *P. brazieri* with both shell size and keel development. For example, in low flow situations, such as Ban Ban Springs in southern Queensland and the Myall Lakes in New South Wales, the shells tend to be a little smaller and keel development tends to not be as conspicuous, whereas in the larger streams and rivers, the shells tend to be larger and have more prominent keels.

Posticobia brazieri is known to act as the intermediate host for at least ten species of



FIGS. 27–30. Details of the snout, cephalic tentacles and penis from critical point dried material of *Posticobia brazier*, NW of Gympie, Queensland (AMS). FIG. 27: Ventral view of snout; FIG. 28: Dorsal view of right cephalic tentacle; FIG. 29: Ventral view of right cephalic tentacle; FIG. 30: Dorsal view of penis. Scale bars B, C = 200 µm; A, D = 100 µm. (Illustrations used with the permission of W. F. Ponder, Australian Museum).

fish trematodes (Cribb, 1985, 1986, 1988a, b). However, the actual number appears to be considerably higher (T. Cribb, personal communication). Infected individuals of *Posticobia* are typically much larger than uninfected individuals and virtually always have very immature / undeveloped reproductive organs, in both males and females (this study).

Posticobia ponderi n. sp.

Figures 1, 5, 11, 15, 21, 25, 36

Diagnosis

Shell small, 1.68–2.60 mm in height, 1.41–1.98 mm in width, conical; body whorl smooth, convex; sutures deeply impressed; keel absent; umbilicus narrow. Operculum with 1–2 weakly developed pegs. Central teeth with 4–5 lateral cusps and 4 pairs of basal cusps.

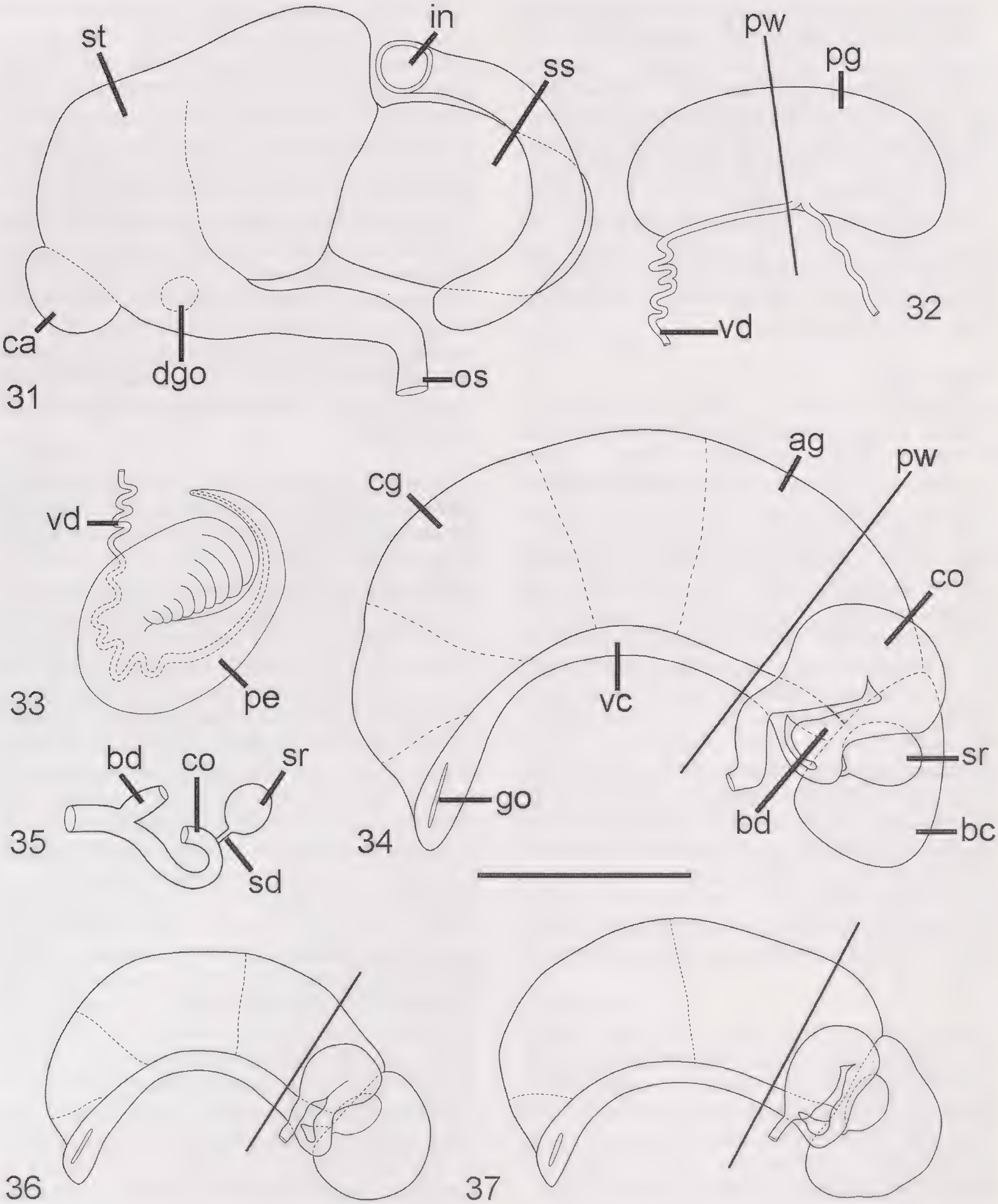
Type Material

Holotype C.461157, paratypes C.203032 (many).

Type Locality

Outflow of small spring, Mulligan Springs, on western edge of Lake Callabonna, 40.1 km E of the Wooltana-Moolawatanna Rd., 29°43’41”S, 139°58’22”E, South Australia, Australia, 0 m, 28 May 1993, in 5–10 mm of water, amongst small sedges, S. A. Clark.

Additional Paratypes – South Australia spring at N end of “Callabonna Peninsula”, Lake Callabonna, 29°43’S, 139°58’E, 19 May 1982 (C.200877); spring on S side of Mulligan Springs, Lake Callabonna, 29°44’01”S, 139°58’E, 20 May 1982 (C.200874); spring on S side of Mulligan



FIGS. 31–37. Illustrations of the stomach, male and female genitalia of *Posticobia* species. FIGS. 31–36: *P. brazieri* north bank of the Clarence River, Grafton, New South Wales; FIG. 31: Stomach; FIG. 32: Prostate gland viewed from the left side; FIG. 33: Penis; FIG. 34: Glandular oviduct viewed from the left side; FIG. 35: Expanded view of the oviduct and seminal receptacle; FIG. 36: Mungo Brush, Myall Lakes, New South Wales; glandular oviduct viewed from the left side *P. chena*; FIG. 37: ***P. ponderi*** Mulligan Springs, Lake Callabonna, South Australia; glandular oviduct viewed from the left side. Scale bar = 0.5 mm.

Springs, near Lake Callabonna, 29°44'01"S, 139°58'01"E, 20 May 1982 (C.200875); southern most spring of Mulligan Springs, Lake Callabonna, 29°44'01"S, 139°58'01"E, 20 May 1982 (C.200876); Mulligan Springs, Lake Callabonna, 29°44'S, 139°58'E, 20 May 1982 (C.200873); spring, 200 m WSW of stn. C209F at N end of "Callabonna Peninsula", Lake Callabonna, 29°45'01"S, 140°00'E, 19 May 1982 (C.200878); spring with pool and swamp, at N end of "Callabonna Peninsula", Lake Callabonna, 29°45'01"S, 140°00'E, 19 May 1982 (C.200879).

Description

Shell (Figs. 5, 11) – Shell 1.68–2.60 mm in height, 1.41–1.98 mm in width, conical in shape; spire of average height, outline convex; sutures deeply impressed. Aperture 0.82–1.20 mm in height, oval, not disjunct from last whorl. Teleoconch of 2.55–3.75 convex whorls, body whorl 1.27–1.85 mm in height, last whorl evenly convex, keel absent. Teleoconch sculpture smooth, with fine growth lines. Inner lip of aperture firmly adhering to parietal wall. Outer lip heavily thickened, lacking an external varix, not reflected. Umbilicus small, narrow in adults, narrowly open in juveniles. Colour opaque, yellowish-brown, periostracum thin, may impart darker colour. Protoconch (Fig. 15) of about 1.25–1.30 whorls; sculptured with dense, irregular shallow pits. Table 1 provides the mean, median and standard deviation of shell dimensions.

Holotype dimensions: SH – 2.18 mm, SW – 1.64 mm, AH – 0.98 mm, BW – 1.64 mm, TW – 3.5.

Operculum – Ovate, thin, flat, yellow, horny, paucispiral, with eccentric nucleus, 0.84–1.08 mm in length, 0.65–0.90 mm in width. White smear 0.17–0.35 mm in length, 0.07–0.19 mm in width; 1–2 weakly developed pegs on inner side. Table 1 provides the mean, median and standard deviation of opercular dimensions.

Radula (Fig. 25) – Central teeth with 4–5 lateral cusps, sharply pointed and about 2 times longer than adjacent cusps; 4 pairs of basal cusps; basal tongue U-shaped, just protruding past lateral edge. Lateral teeth with 5 cusps on each side, sharply pointed, about 2 times longer than adjacent cusps; basal projection U-shaped, lateral flange longer than cutting edge. Inner marginal teeth with 23–24 cusps. Outer marginal teeth with 27–30 cusps.

Head-Foot – Animal unpigmented.

Mantle Cavity – Ctenidium with 19–24 triangular filaments, width at widest point 0.15–0.20 mm, apices near right side, posterior end abutting pericardium. Osphradium 0.22–0.36 mm in length, white, elongately oval, located between posterior end and middle of ctenidium. Hypobranchial gland inconspicuous to well developed, partly to completely covering rectum. Rectum straight or with weakly developed arch (arch more developed in males). Visceral coil: Renal organ extending forward ca. 1/2 into pallial cavity, orientated longitudinally. Renal gland 0.28–0.43 mm in length and 0.17–0.27 mm in width. Pericardium more than 1/2 in pallial roof.

Stomach – Stomach 0.49–0.67 mm in length, style sac 0.29–0.40 mm in length, with very small gastric caecum 0.06–0.07 mm in length.

Male Reproductive System – Testis occupying about 1.0–1.3 whorls. Seminal vesicle beneath anterior 0.30–0.40 of first whorl of testis behind stomach, loosely to tightly coiled over stomach; conspicuously coiled on both digestive gland and testis behind stomach. Prostate gland 0.40–0.49 mm in length, 0.17–0.18 mm in width, about 1/2 in pallial roof, kidney-shaped, oval to compressed-oval in section. Pallial vas deferens flush with surface of neck and slightly undulating at prostate gland, strongly undulating between prostate and base of penis. Penis unpigmented, distal part short, tapering, base broad, creased (when at rest). Penial duct strongly undulating in base, straight in distal portion of penis. Penis located just to right of midline, 0.28–0.34 mm behind right eye.

Female Reproductive System (Fig. 36) – Ovary occupying about 0.75–1.00 whorls. Coiled oviduct with initial U-shaped bend orientated obliquely backwards; initial loop medium, proximal part simple; distal part straight to seminal receptacle. Coiled oviduct and bursal duct joining just behind pallial wall, with oviduct joining bursal duct from right side. Oviduct straight, before joining with bursal duct. Bursal duct 0.04–0.08 mm in length, 0.10–0.11 mm in width, parallel sided, straight. Bursa copulatrix 0.22–0.36 mm in length, 0.16–0.25 mm in width. Seminal receptacle located on or near anterior edge of bursa copulatrix to towards middle of inner wall of bursa copulatrix. Seminal receptacle 0.09–0.13 mm in length, 0.07–0.12 mm in width, pyriform in shape to a narrow sac with short duct. Rectum overlapping both albumen and capsule glands. Albumen gland 0.22–0.34 mm in length, 0.22–0.29 mm in width, more than 1/2 of albumen gland in front of posterior pallial wall. Capsule gland 0.31–0.45 mm in length, 0.21–0.28 mm in width, with anterior end tapering to blunt, oval to compressed-oval in section, with distinct glandular zones. Ventral channel indistinct to distinct, with indistinct to moderate-sized muscular vestibule. Genital opening small to medium in length, overlapping anterior end of capsule gland.

Egg Capsules – Egg capsules very similar in size and shape to those observed for *P. brazieri* and found on similar substrata.

Distribution

Known from a number of small springs that make up the Mulligan Springs complex on the western edge of Lake Callabonna. The species is abundant but sporadically distributed among the springs in two discreet areas.

Etymology

Named for my friend and mentor Winston Ponder, in recognition of his extensive research on Australian molluscs, particularly the family Hydrobiidae over more than 30 years. He was the first to find the Mulligan Springs populations and highlighted their distinctiveness to the author.

Remarks

The species is currently known only from a small number of springs, with additional surveys in the area are likely to find additional populations. ***Posticobia ponderi*** is generally smaller than the other two species; it only has 1–2 weakly developed pegs on the inner side of the operculum and the prostate gland is half in the pallial cavity. However, probably the most distinctive character is the total lack of a keel or shoulder on the body whorl of ***P. ponderi*** compared to the other species. ***Posticobia ponderi*** is most similar in terms of general shell shape to populations of *P. brazieri* from the Roper River in the Northern Territory (Figs. 10, 11).

Discriminant function analysis using only shell measurements (SH, SW, AH, BW, CV, TW) separated all three taxa (Wilks' lambda = 0.632; Approx. F = 19.619, df = 10,760 p-tail < 0.000) with only 6 of 30 specimens of ***P. ponderi*** misidentified as *P. brazieri* for an overall classification rate of 80%.

Posticobia norfolkensis (Sykes, 1900)

Figures 1, 6, 12, 16, 22, 26

Paludestrina norfolkensis Sykes, 1900: 146, pl. 13, fig. 14, Norfolk Island.
Posticobia norfolkensis – Ponder, 1981: 18–28, fig. 2, 1–5; Smith, 1992: 54.

Type Material

Syntypes NHML 1856.10.27.94 (32 specimens).

Type Locality

Norfolk Island, about 1,400 km E of Brisbane, New South Wales, Australia.

Material Examined

New South Wales Bumbora Beach, Norfolk Island, 29°03'40"S, 167°57'30"E, 1909 (C.30992); Norfolk Island, 29°03'30"S, 167°57'30"E (C.34522); Bumbora, Norfolk Island, 29°03'40"S, 167°57'30"E, 4 Aug. 1913 (NMNZ FM20070).

Diagnosis

Shell 1.70–2.71 mm in height, 1.50–2.35 mm in width, ovate; sutures moderately impressed; last whorl sub-shouldered, convex, keel absent; umbilicus narrow. Protoconch of about 1.25 whorls, sculptured with sparsely distributed irregular shallow pits. Operculum yellow? with 3 weakly developed pegs. Central teeth with 5 lateral cusps, 4 pairs of basal cusps.

Description

Shell (Figs. 6, 12) – Shell 1.70–2.71 mm in height, 1.50–2.35 mm in width, ovate in shape, spire of average height, outline convex, sutures moderately impressed. Aperture 0.89–1.37 mm in height, oval, not disjunct from the last whorl. Teleoconch of 2.40–3.50 convex whorls, body whorl 1.38–2.15 mm in height, last whorl sub-shouldered, convex, keel absent. Inner lip of aperture slightly separated from parietal wall. Outer lip heavily thickened, lacking external varix, not reflected. Teleoconch sculpture smooth, with fine growth lines. Umbilicus narrow. Colour yellowish-brown, periostracum thin. Protoconch (Fig. 16) of about 1.25 whorls, sculptured with sparsely distributed, irregular shallow pits. Table 1 presents the mean, median and standard deviation of shell dimensions.

Operculum (Fig. 22) – Ovate, thin, flat, yellow, horny, paucispiral, with eccentric nucleus, with up to 3 weakly developed pegs on inner side.

Radula (Fig. 26) – Central teeth with 5 lateral cusps, 4 pairs of basal cusps; sharply pointed, about 2 times longer than adjacent cusps; basal tongue U-shaped, just protruding past lateral edge. Lateral teeth with 5 cusps on each side, sharply pointed, about 2 times longer than adjacent cusps; basal projection U-shaped, lateral flange longer than cutting edge. Inner marginal teeth with 24–26 cusps. Outer marginal teeth with 35–38 cusps.

Details of the living animal, its internal anatomy and egg capsules unknown.

Distribution

Known only from the type locality (Fig. 1).

Remarks

Due to the limited material available of *P. norfolkensis*, it is tentatively recognised as a separate species of *Posticobia*. Unfortunately, this species is believed to be extinct, as a considerable effort was made to recollect it without success in June 1979 by Winston Ponder and November 1997 by Robert Varman. Habitat destruction due to human intervention and damage caused by grazing cattle were indicated as the major contributors to the demise of this species (Ponder, 1981). However, R. Varman has suggested that a combination of clearing, grazing, and a devastating flood locally known as "The Flood", which occurred around 1936, may have significantly affected the species' survival (personal communication).

Another possibility is that the species was not actually endemic to Norfolk Island but may represent a transient population of *P. brazieri* from the Australian mainland, as the only known material of this species was collected from a small stream flowing through what is now known as Bumbora Reserve.

Discriminant function analysis using only shell measurements (SH, SW, AH, BW, CV, TW) separated all three taxa (Wilks' lambda = 0.632; ca. F = 19.619, df = 10,760 p-tail < 0.000), with only 1 of 20 specimens of *P. norfolkensis* misidentified as *P. brazieri* for an overall classification rate of 95%.

Posticobia sp.

?*Austropyrgus* species, Clark et al., 2003: 104.

Remarks

Clark et al. (2003) illustrated (Fig. 63: A1–A3) specimens of a population collected from a sinkhole near Mt. Gambier, South Australia, which are considered to be an unusual ecological variant of *P. brazieri*. These specimens are typical of *P. brazier*, except that the body whorl has separated from the rest of the shell. The population co-occurs with *Austropyrgus vastus* Clark et al., 2003, and to date no living material of either form has been located, and thus the true affinities of this population remain unclear.

DISCUSSION

Posticobia is genetically and anatomically closely related to *Austropyrgus* (Clark, 1997; Perez et al., 2005). Despite this, the two genera show markedly different patterns of speciation. *Posticobia* has one very wide ranging species, with two additional species occurring in isolated populations on the eastern and western peripheries of the distribution of the genus, whereas *Austropyrgus* has over 70 described species and many more undescribed, the majority of which, have small to very small ranges (Clark et al., 2003). This is probably due in part to the differing preferred habitats for the two genera. A similar situation is seen with the New Zealand genus *Potamopyrgus,* with one wide ranging species and a small number of additional species with very narrow ranges (Winterbourn, 1970; Haase, 2008).

Posticobia tends to live in large coastal rivers, streams and lakes (except for ***P. ponderi*** very small springs), which, are relatively open and allow access to such passive dispersal agents as birds (Boag, 1986; Ribi, 1986), fish (Haynes et al., 1985), and floods. *Austropyrgus* tends to live in small streams and springs with closed canopies and little access to passive dispersal agents (Ponder et al., 1994; Ponder & Colgan, 2002). Allozyme genetic variation both within and among populations is considerably higher in *Austropyrgus* than in *Posticobia* (Clark, 1997), implying that gene flow is higher in *Posticobia*, which appears to be related to habitat differences (Ponder et al., 1994). Thus, predominantly due to habitat preference and restricted gene flow, *Austropyrgus* is much more speciose, and species have much more restricted distributions than *Posticobia* (Clark et al., 2003).

An interesting aspect of the distribution of *Posticobia* is the large gap between the populations found in Sydney, New South Wales, to those found in far western Victoria and southeastern South Australia (Fig. 1). Within this region, populations of *Austropyrgus* are found in virtually all suitable streams from southern New South Wales south and west along the coastal regions to southeastern South Australia, with some streams and springs having up to four sympatric species and frequently occur in very high densities (Clark et al., 2003). There are a few possible explanations for this including: (i) that *Posticobia* is currently expanding its range, especially into western Victoria and southeastern South Australia, as the known populations from this general area are relatively widely scattered (although this could just be an artifact of sampling effort); (ii) that the more isolated populations of *Posticobia* in South Australia and Victoria are remnant populations of a single formerly more widespread species that has undergone range contraction via recent extinction events due to such factors as habitat loss/modification or increased aridity in the intermediate areas; (iii) that these isolated population represent additional cryptic species; (iv) that *Posticobia* is essentially outcompeted by *Austropyrgus*; this is based on limited observations in South Australia where the two genera are sympatric, *P. brazieri* was less numerous than *A. macropus* at a site on the Wakefield River, N of Adelaide; or (v) that as the invasive New Zealand hydrobiid *Potamopyrgus antipodarum* continues to expand its range in southeastern Australia, it can more rapidly colonise new/available habitats than can *Posticobia*. Future molecular studies could provide useful information towards resolving hypotheses (i–iii). However, hypotheses (iv–v) are more speculative and are based on limited observations, but nonetheless it would be interesting to determine if there are any competitive interactions between sympatric species in different genera or indeed between sympatric species in the same genus. I know of no studies of this kind for native Australian hydrobiids, although there has been some work looking at interactions between *Potamopyrgus antipodarum* stream disturbance and native aquatic invertebrates (Schreiber et al., 2003).

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THE POTENTIAL OF THE INVASIVE SNAIL *POMACEA CANALICULATA* AS A PREDATOR OF VARIOUS LIFE-STAGES OF FIVE SPECIES OF FRESHWATER SNAILS

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ABSTRACT

The invasive apple snail *Pomacea canaliculata* is known as an omnivorous species, but there are only few reports of its predation. This study examined the potential of this snail as a predator of common freshwater snails in southern China. Laboratory experiments were conducted to quantify the damage of the apple snail to both early stages (eggs and/or neonates) and adults of five species of freshwater snails (*Austropeplea ollula*, *Biomphalaria straminea*, *Melanoides tuberculata*, *Physa acuta*, *Sinotaia quadrata*). The apple snail caused significant mortality to all of the early stages of the five snails, as well as adults of the pulmonates *A. ollula*, *B. straminea* and *P. acuta*, but did not consume adults of the prosobranchs *M. tuberculata* and *S. quadrata*. Such differential survival of the prey might be explained by differences in shell hardness and structure, as the adult prosobranchs were well protected by a hard shell and an operculum, whereas the pulmonates had a relatively fragile shell and lacked an operculum. The apple snail was unable to detect its prey from a distance, but it crawled quickly, which could create opportunities for direct contact with potential prey. Apple snails may therefore influence invaded ecosystems through predation on other freshwater snails.

Key words: apple snail, invasive species, crushing resistance, *Pomacea*, predation.

INTRODUCTION

The golden apple snails, *Pomacea canaliculata* (Lamarck, 1822) and *P. insularum* (d'Orbigny, 1835) (Mollusca: Ampullariidae), are native to the freshwater wetlands of South America, but have been widely introduced into Asia and into North and Central America since the 1980s as aquarium pets or human food (Cowie, 2002; Estebenet & Martín, 2002; Joshi & Sebastian, 2006; Rawlings et al., 2007; Hayes et al., 2008). These species possess several traits of successful invasive species. Under favorable conditions, they can reach a life-span of up to four years (Estebenet & Cazzaniga, 1992), attain maturity in 2 to 4 months (Estoy et al., 2002), and each female can lay up to 8,680 eggs per breeding season (Miyahara et al., 1986). They are highly adaptable to harsh environmental conditions, such as low dissolved oxygen concentration, high nutrient content, low food supply, and low temperature (Cowie, 2002).

In its invaded regions, *Pomacea* spp. are mainly known as agricultural pests. They are voracious feeders on wetland crops, causing tremendous loss to the rice paddies of Asia and taro farms of Hawaii (Naylor, 1996; Halwart, 1994; Cowie, 2002; Joshi & Sebastian, 2006). Apart from crops, these species have also been reported to feed on wild macrophytes. For example, Estebenet (1995) and Lach et al. (2000) conducted laboratory experiments to study the feeding of *P. canaliculata* on some common aquatic macrophytes in Argentina and Hawaii, respectively. They found that the snails exhibited a clear preference for certain macrophytes, and that their growth rates were high when fed with the preferred macrophytes. In the field, Carlsson et al. (2004) found high densities of *P. canaliculata* to be associated with low diversities of aquatic macrophytes, high nutrient concentrations, and high phytoplankton biomass in wetlands. Herbivory by apple snails caused a loss of wild macrophytes (Carlsson et al., 2004; Carlsson & Lacoursière, 2005), and a change towards dominance by phytoplankton (Carlsson et al., 2004).

Previous studies have thus highlighted the important herbivorous impact of *Pomacea*

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canaliculata on crop production, wetland floral diversity, and ecosystem functioning. However, relatively few studies have been conducted to determine the impact of these invasive gastropods on other aquatic invertebrates. Cazzaniga (1990) reported predation by *P. canaliculata* on eggs, neonates, and adults of the gastropod *Biomphalaria peregrina*. Cha (1989) reported predation of *P. canaliculata* on eggs and neonates of *Biomphalaria straminea* and on the neonates of three other snail species (*Austropeplea ollula, Physa acuta, Melanoides tuberculata*), but no predation on the neonates of another snail (*Sinotaia quadrata*). Wood et al. (2006) reported that *P. canaliculata* grazed actively and selectively on freshwater bryozoans. Nevertheless, these studies were conducted without offering food to the predator before the experiment (Cha, 1989) or during the experiment (Cazzaniga, 1990; Wood et al., 2006). It remains unknown whether non-starved *P. canaliculata* will prey on other invertebrates when alternative food is available, how the predator detects its prey and what determines the vulnerability of the prey.

This study examined the potential of *Pomacea canaliculata* as a predator of the early stages as well as adults of five species of snails commonly found in the freshwater wetlands of southern China. Each species of potential prey was kept with *P. canaliculata* for a certain period of time in laboratory aquaria, after which prey mortality was recorded. To understand whether shell hardness plays a role in the relative vulnerability of the potential prey, their crushing resistance was determined and a relationship between shell length and crushing resistance established for each species. To understand the mechanism underlying prey selection, tests were conducted to examine the crawling behaviour of *P. canaliculata* and determine whether it could detect prey from a distance.

METHODS

Potential Prey Species

The experiments were conducted between February 2007 and April 2008 at Hong Kong Baptist University (HKBU). Five species of snails were used: *Austropeplea ollula* (Gould, 1859) (Lymnaeidae), *Biomphalaria straminea* (Dunker, 1848) (Planorbidae), *Physa acuta* Draparnaud, 1805 (Physidae), *Melanoides tuberculata* (Müller, 1774) (Thiaridae), and *Sinotaia quadrata* (Benson, 1842) (Viviparidae). These species are common in freshwater wetlands of southern China. The first three species are pulmonates, whereas the last two are prosobranchs. The three pulmonates are oviparous, laying submerged egg masses. The two prosobranchs are ovoviviparous, retaining eggs and developing embryos in the body, and giving birth to free-crawling juvenile snails. For the oviparous species, tests were performed on the eggs masses, neonates, and adults. For the ovoviviparous species, tests were performed on the neonates, juveniles and adults of various sizes.

Collection and Maintenance of Test Animals

Juveniles and adults of the potential prey were collected from various shallow water environments (semi-aquatic vegetable farms, irrigation channels, and ponds) in the New Territories, Hong Kong. They were reared at Hong Kong Baptist University in separate stock aquaria containing 50 L dechlorinated tap water at 22 ± 1°C. Aeration was provided to each aquarium with an air pump via plastic tubing and an air stone. Approximately 1/3 of the water was changed twice a week to avoid fouling. A commercial fish meal was fed *ad libitum* to the snails three times a week. Leaves of romaine lettuce (*Lactuca sativa var. longifolia*) were also supplied to the species that feed on plants (*A. ollula, B. straminea* and *P. acuta*). Under these conditions, the snails grew and reproduced successfully. Juveniles and adults of the potential predator, *P. canaliculata*, were collected from an irrigation channel in Yin Kong Village, the New Territories, and reared in an aquarium containing 80 L dechlorinated tap water and fed with romaine lettuce. All other rearing conditions for *P. canaliculata* were the same as those for the potential prey species.

Experiment 1. Predation of *P. canaliculata* on Eggs, Juveniles and Adults of the Potential Prey

For each prey species, tests of predation by the apple snail on a particular life stage were run using three aquaria, each containing 8 L dechlorinated tap water. Three aquaria with the prey but without *P. canaliculata* were used as experimental controls. Two size classes of apple snails (12–15 mm and 37–40 mm shell length) were tested separately. Since this apple snail becomes mature at around 25 mm shell length (Estebenet & Martín, 2002; Estoy et al., 2002), the smaller individuals were juveniles and the larger individuals were adults. The experimental temperature was the same as that used in rearing the juveniles and adults. In addition to the following quantitative tests, direct observation of the predatory behavior of *P. canaliculata* was also conducted in separate aquaria.

In tests of predation on egg masses of the three pulmonate species, 20 to 40 adults of a prey species were transferred from the stock aquarium into each of the experimental aquaria and kept for up to three days. When each aquarium contained more than 25 egg masses, the adults were removed. The egg masses of these species are gelatinous, ranging from 3.5 to 5.5 mm in diameter, each containing 15 to 30 embryos. They were laid on the bottom and walls of the aquaria, and were visible with the naked eye. After marking the positions of these egg masses, an individual of *P. canaliculata* was introduced into each aquarium. To determine if there was an ontogenetic shift in predation, both juvenile and adult *P. canaliculata* were used in separate tests. Romaine lettuce was provided *ad libitum*, and the aquaria were covered with an opaque plastic bag to avoid directional effect of light on snail movement. To avoid the potential effect of laboratory predatory experience on predation rate, each individual of *P. canaliculata* was used only once. After 24 h, the aquaria were inspected and the numbers of egg masses remaining intact, that were damaged, and that had disappeared were recorded. Preliminary observation had shown that the egg masses would remain intact and attached to the bottom/walls of the aquaria until the juveniles hatched. Since it took at least five days for the embryos to develop into juveniles and hatch (Kwong, unpublished data), predation was considered to be the cause of disappearance of egg masses.

In tests of predation on neonates (i.e., individuals hatched within the previous 24 h), each aquarium contained 60 ± 5 neonates. The neonates of the three pulmonates were of similar sizes, with approximately 1 mm maximum dimension (shell width or shell height). The neonates of the two prosobranchs were similar in size (approximately 3 mm shell length) but much larger than those of the pulmonates. Three individuals of *P. canaliculata* were introduced into each test aquarium. After 72 h, the numbers of neonates alive, dead and that had disappeared were recorded, and the shells examined for signs of attack.

In tests of predation on adults, 15 individuals of a potential prey species were introduced into each aquarium together with three individuals of *P. canaliculata*. The size range of the prey was 7–8 mm shell width (SW) for the disc-shaped *B. straminea*, and 13–15 mm shell length (SL) for *A. ollula*, 10–12 mm for *P. acuta*, 23–26 mm for *M. tuberculata* and 25–30 mm for *S. quadrata*, all of which are species with tall, narrow shells. Although different in size, they were all relatively large mature individuals of the respective species. Two whole leaves of romaine lettuce (fresh weight 30 ± 5 g) and 30 mg of fish meal (Hakari® Algal Wafer) were provided as food for the snails during the experiment. In 24 h, apple snails consumed less than half of food offered. Excessive food was removed, and new food was offered daily. After 72 h, the number of dead individuals in each aquarium was recorded and the shells examined for signs of attack.

Experiment 2. Size-dependent Predation of *P. canaliculata* on Two Prosobranchs

Experiment 1 showed that adult *P. canaliculata* preyed upon the neonates of *M. tuberculata* and *S. quadrata*, but not on the large adults of these prosobranchs (see Results). This follow-up experiment examined whether apple snails could prey on the juveniles and smaller adults of the two prosobranchs. In total, 111 *M. tuberculata* and 84 *S. quadrata* were used. These individuals ranged from neonates to large adults (3–28 mm SL). For each prey species, all individuals were introduced into an aquarium containing 25 L water and 10 adults of *P. canaliculata* (35–40 mm SL). Fish meal and romaine lettuce were fed to the snails *ad libitum*. Water was changed daily to avoid fouling. After 3 days, living prosobranchs were collected from each aquarium and their shell lengths measured. The shell length frequency data before and after the experiment were compared.

Experiment 3. Crushing Resistance of Different Prey Species

The crushing resistance of the five species of prey was determined using a mechanical crusher. This device consists of a heavy duty lab jack, an electronic balance (maximum weight: 10 kg, precision: ± 0.001 kg) for measuring the crushing strength, and a stainless steel vice for holding the specimens. The whole rig was mounted vertically and vertical movement was achieved by a rotary mechanism on the jack. During measurement, a live prey individual was placed in the vice and the crushing weight read directly from the electronic balance. For the disc-shaped *B. straminea*, force was directed through the body whorl perpendicular to the periphery of the shell. For the other four species, force was directed along an axis perpendicular to the columella (Lewis & Magnuson, 1999). The vice was tightened by the jack until the shell was crushed. During this process, the force was gradually increased up to the sudden event of shell collapse. The maximum reading was taken as the crushing resistance. For each species, the measurements covered a size range from young juveniles to large adults (3.0–17.5 mm SL for *A. ollula*, n = 69; 2.0–5.7 mm SW for *B. straminea*, n = 49; 2.4–10.0 mm SL for *P. acuta*, n = 63, 3.2–19.0 mm SL for *S. quadrata*, n = 83; and 3.0–28.0 mm SL for *M. tuberculata*, n = 77). However, the smallest pulmonates (≤ 2 mm SL or SW) were too fragile to be measured using this method. The data were used to establish a relationship between shell width/length and crushing resistance for each prey species.

Experiment 4. Prey Detection and Crawling in *P. canaliculata*

Assays of prey detection by *P. canaliculata* were performed using a circular plastic basin (diameter 60 cm, depth 10 cm). With six equally spaced acrylic plates extending 20 cm from the wall to the center, the basin was partitioned into six fan-shaped side compartments and a central circular compartment. Three alternate side compartments were randomly assigned as replicates, each containing the prey species. A fixed number of adults of one prey species (15 individuals of *A. ollula*, *B. straminea*, *M. tubeberculata* and *P. acuta*, and eight individuals of *S. quadrata*) were confined in a paper tea bag (10 x 7 cm) with twenty 3 mm diameter pores. The other three side compartments each contained an empty tea bag and served as controls. Twenty adult *P. canaliculata* (35–40 mm SL) were introduced into the central compartment. The apple snails were allowed to move towards the side compartments. The basin was covered with a lid to prevent any directional effect of light on snail movement. After 30 minutes, the number of snails in each compartment was recorded. A comparison of the total number of snails in the control and test compartments gave a relative prey preference. The assay for each species was conducted three times.

Assays were also conducted to examine the crawling behavior of *P. canaliculata* using a rectangular (44 L x 58 W x 14 H cm) plastic tray. The tray contained a layer of clay (1 cm) collected from a semi-aquatic vegetable garden in Long Valley, the New Territories. Over the clay was 4 cm of dechlorinated tap water. The assays were conducted during the night in almost complete darkness when the apple snail was most active. Snail movement was observed with the aid of a small torch. In each assay, one adult apple snail (3.5–4.0 cm SL) was introduced into the centre of the box and its movement traced for a one-hour period, starting from the time when its muscular foot became fully extended. As the snail moved, it left a track on the clay. The track was drawn on a piece of paper and the total distance travelled measured afterwards. The 1 h assay was repeated nine times using different individuals. The water and mud were changed between assays to avoid the potential effect of snail mucus on snail movement.

Data Analysis

For each species, the effects of predator size (juvenile or adult) and prey species on consumption of egg masses, neonates or adults were assessed using two-way analysis of variance (ANOVA). The relationship between shell size and crushing weight for each prey species was established using simple linear correlation or a 2-parameter exponential growth equation, based on visual observation of the data. For each species of prey, the total number of predators (i.e., apple snails) in the controls and that in the experimental compartments were compared using a paired *t*-test, with the three assays being used as replicates.

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FIG. 1. Predation of *Pomacea canaliculata* on other snails. A: Adult apple snail approaching an egg mass of *Physa acuta*. The egg mass was consumed by the predator approximately 1 minute after; B: Neonate *Melanoides tuberculata* showing a piece of flesh in the missing posterior shell; C: Neonate *Sinotaia quadrata*, showing the empty shell without an apex; D: Empty intact shell of an adult *Austrapeplea ollula*; E: Empty intact shell of an adult *Physa acuta*; F: Adult *Biomphalaria staminea*, showing the broken shell and some unconsumed soft body. Scale bars A = 1 cm; B, C = 1.5 mm; D–F = 5 mm.

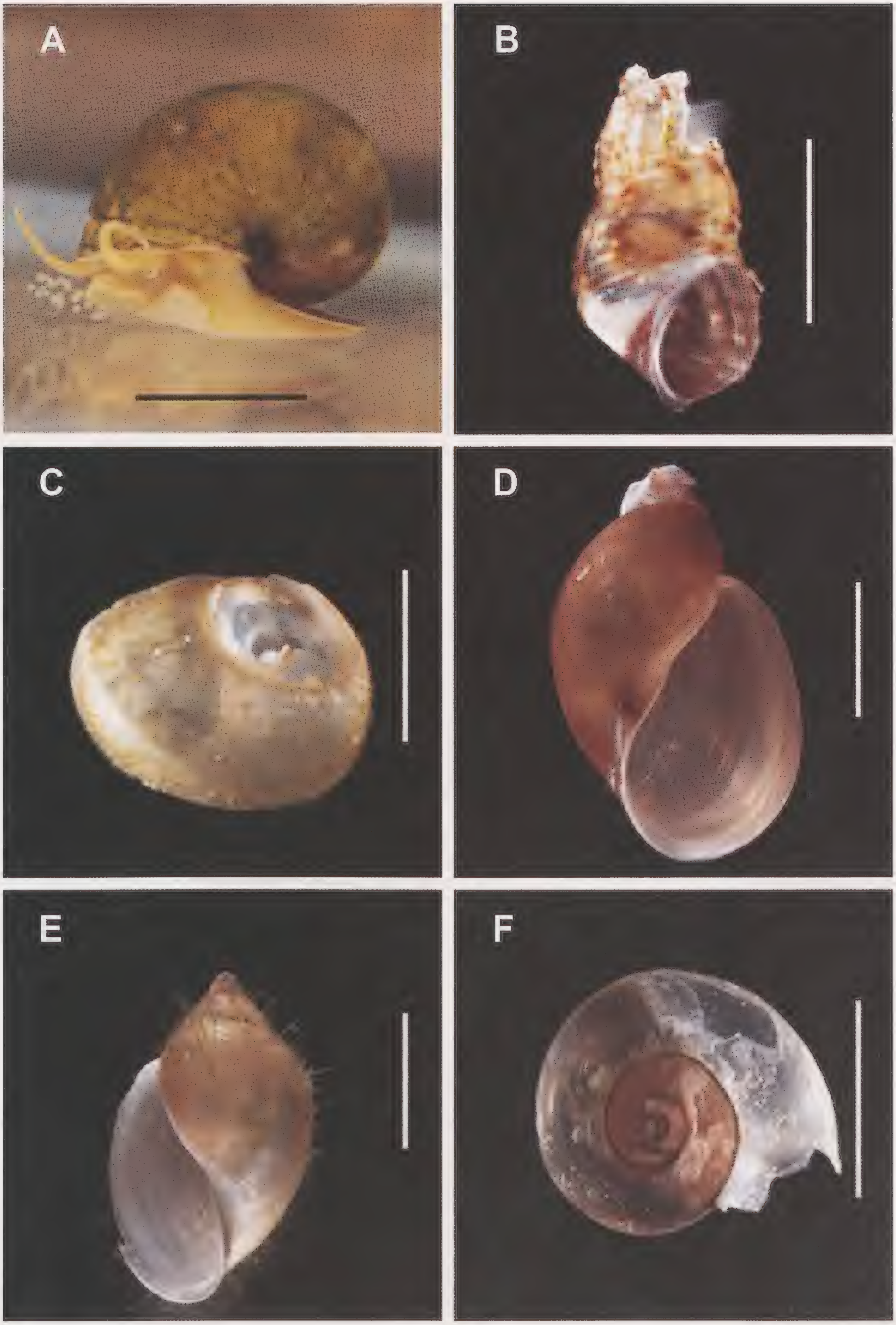
A
B
C
D
E
F

TABLE 1. Predation of *Pomacea canaliculata* on different species of prey snails. Two size classes of the predator were used. Separate tests were conducted with egg masses, neonates and adults of the preys. Two-way ANOVA was used to test effects of prey species and predator size (adult vs. juvenile) on percentage of predation. NS: not significant.

Life stage of prey	*df*	*F*	*P*
Experiment 1A: Egg masses			
Prey species (A)	2	32.62	< 0.0001
Predator size (B)	1	2.089	0.1665 (NS)
A x B	2	0.2206	0.8053 (NS)
Experiment 1B: Neonates			
Prey species (A)	4	24.47	< 0.0001
Predator size (B)	1	78.98	< 0.0001
A x B	4	15.30	< 0.0001
Experiment 1C: Adults			
Prey species (A)	4	4.122	0.0135
Predator size (B)	1	23.67	< 0.0001
A x B	4	4.122	0.0135

RESULTS

Experiment 1. Predation of *P. canaliculata* on Eggs, Juveniles and Adults of Potential Prey

Apple snails were observed to approach and engulf prey egg masses (Fig. 1A). Although on a few occasions part of the gelatinous matrix coating the eggs remained, the embryos were always removed. In control aquaria, all egg masses remained intact during the course of the experiment. Adult and juvenile *P. canaliculata* consumed 69–85% and 42–52% of the egg masses, respectively (Fig. 2A). The predators significantly reduced the number of egg masses, and the consumption rate of adults was significantly higher than that of juveniles (Table 1). The egg masses of the three prey species did not differ significantly in their susceptibility to predation.

In the controls, all neonate prey survived through the experiment. In the experimental treatments, adult and juvenile *P. canaliculata* consumed 22–76% and 8–48% of the neonates of the prey, respectively (Fig. 2B, Table 1). The pulmonates (31–76%) in general suffered a higher predation rate than the prosobranchs (8–42%). Adult apple snails had a significantly higher consumption rate than juveniles (Table 1). The neonates of the pulmonates (*A. ollula*, *B. straminea* and *P. acuta*) were usually entirely engulfed, without leaving hollow shells or shell fragments. In contrast, shells of the prosobranch neonates were broken, with either some tissues remaining, as in *M. tuberculata* (Fig. 1B), or completely missing, as in *S. quadrata* (Fig. 1C).

All adult prey in the controls survived through the experiment. In the treatments, juvenile *P. canaliculata* did not consume any adult prey (Fig. 2C). The adult apple snails, however, consumed the adult pulmonates, but the consumption rate was low (33% of *A. ollula*, 24% of *P. acuta* and 27% of *B. straminea*) and not significantly different among prey species. All adult prosobranchs survived through the experiment. Examination of the dead pulmonates revealed different attack patterns. In *A. ollula* and *P. acuta*, the soft body was usually missing, but the shell was intact (Fig. 1D, E). In *B. straminea*, the shell opening and occasionally the outermost whorl were broken, with parts of the soft body, especially the head and foot, missing; the visceral mass usually remained (Fig. 1F).

Experiment 2. Size-dependent Predation of *P. canaliculata* on Two Prosobranchs

In the controls, all individuals of the two prosobranch species survived through the experiment. In the experimental treatments 14.3% of the *S. quadrata* and 12.6% of the *M.*

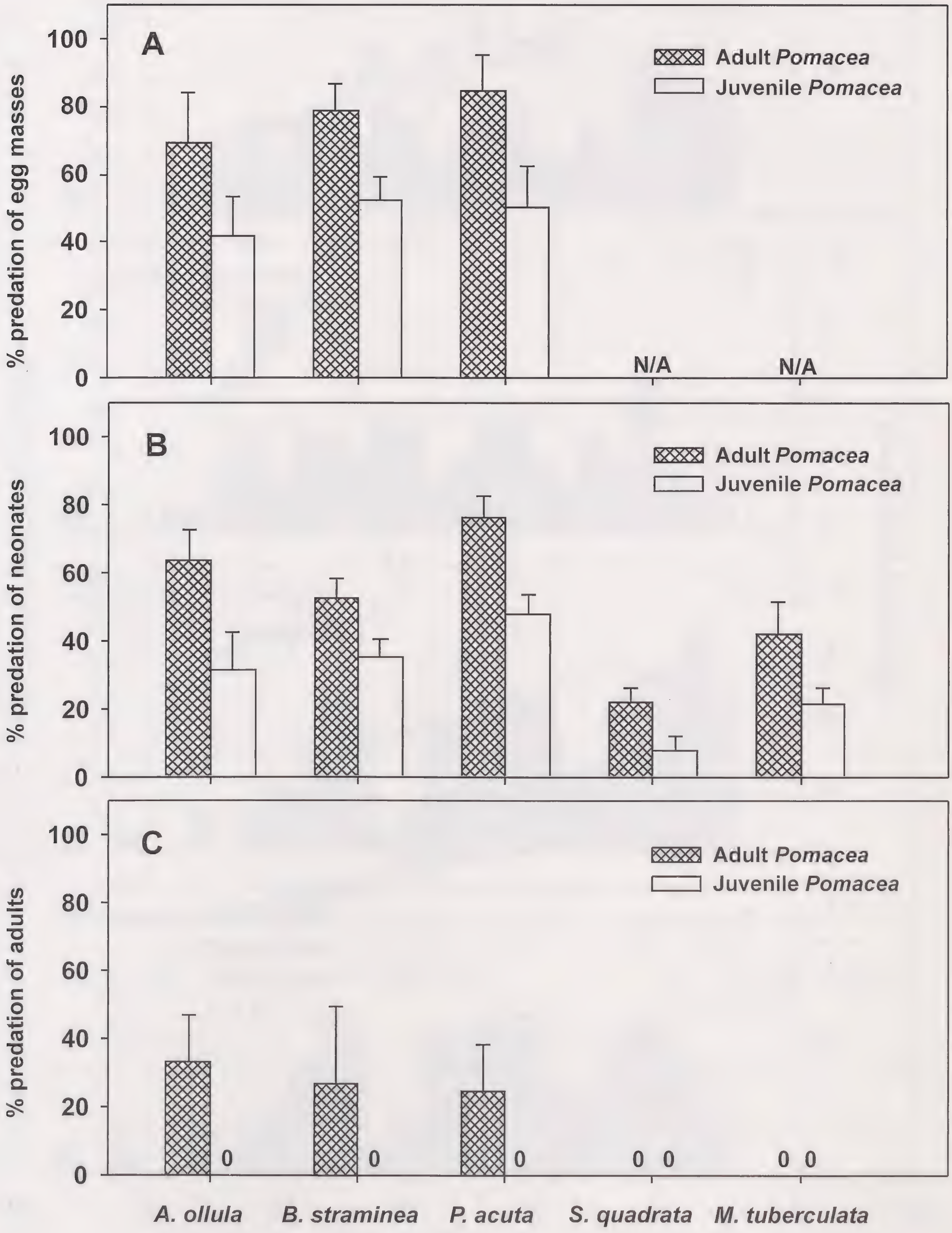

FIG. 2. Predation rate of adult (shaded bars) and juvenile (open bars) *Pomacea canaliculata* on other snails. A: Egg masses of three species of pulmonates. Data are not available (N/A) for the two species of prosobranchs, which do not lay eggs; B: neonates of five species of preys; C: adults of five species of preys. A zero represents no predatory mortality in the treatment. Each bar represents the mean of 3 replicate aquaria ± S.D.

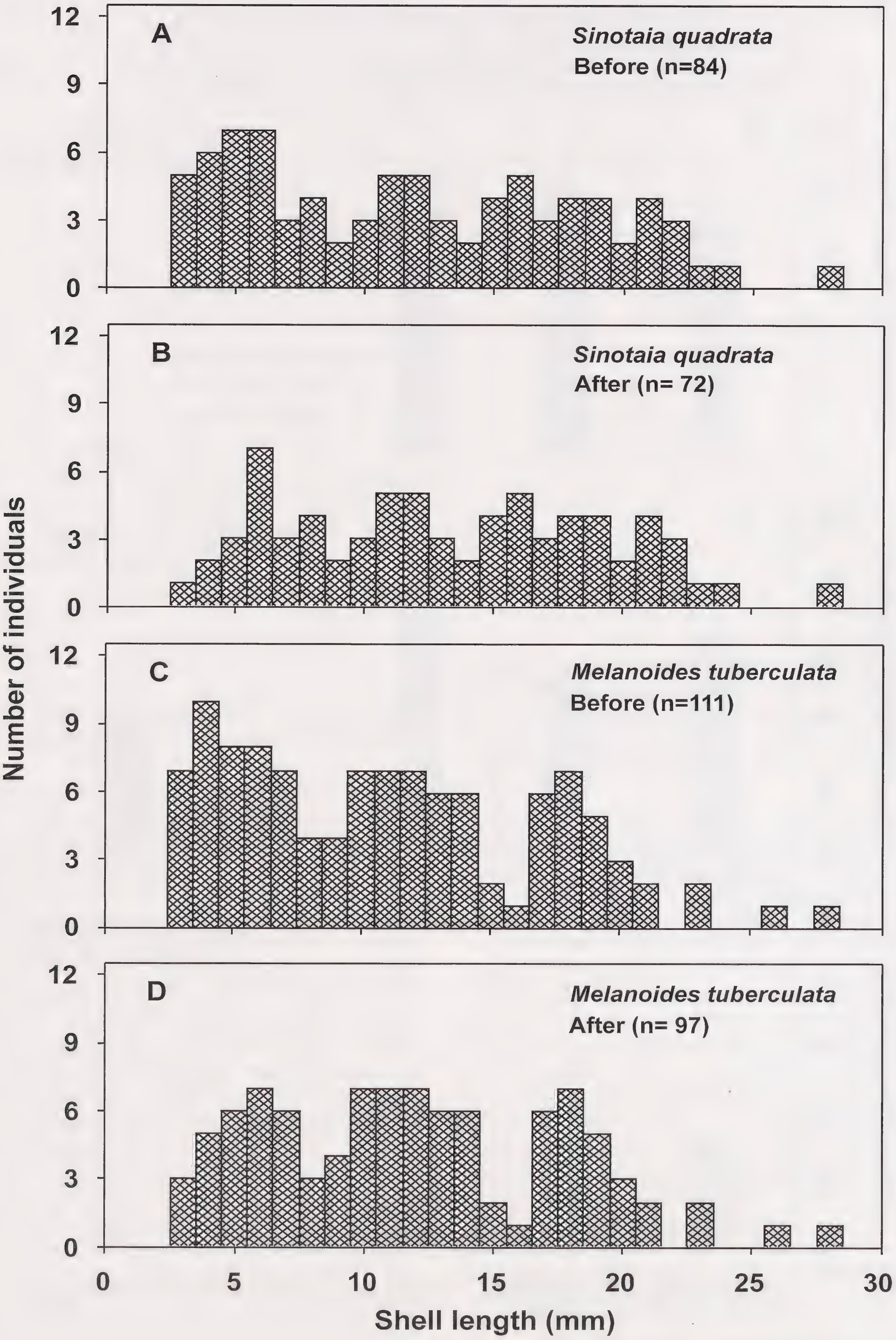

FIG. 3. Size-dependent predation of adult *Pomacea canaliculata* on the two prosobranchs. A, B: Represents the number of surviving *Sinotaia quadrata* before and after 72-h contact with the predator, respectively; C, D: Represents the number of surviving *Melanoides tuberculata* before and 72-h after contact with the predator, respectively.

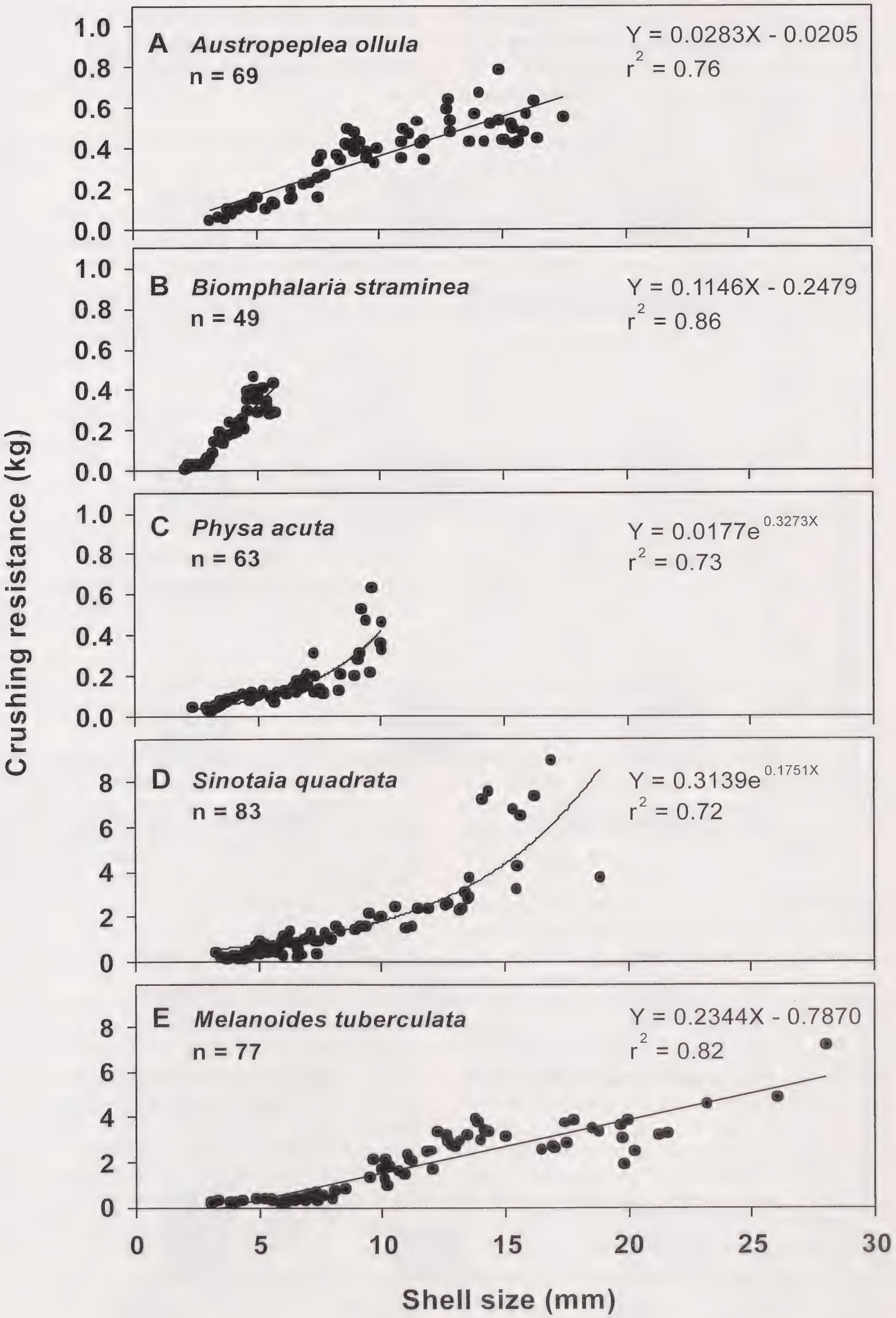

FIG. 4. Size-dependent crushing resistance in the five species of preys. The shell size refers to shell length, except for *Biomphalaria straminea*, which is shell width. The maximum adult sizes are very different among the species. Note that the scale of Y axis for the pulmonates is different from that for the prosobranchs.

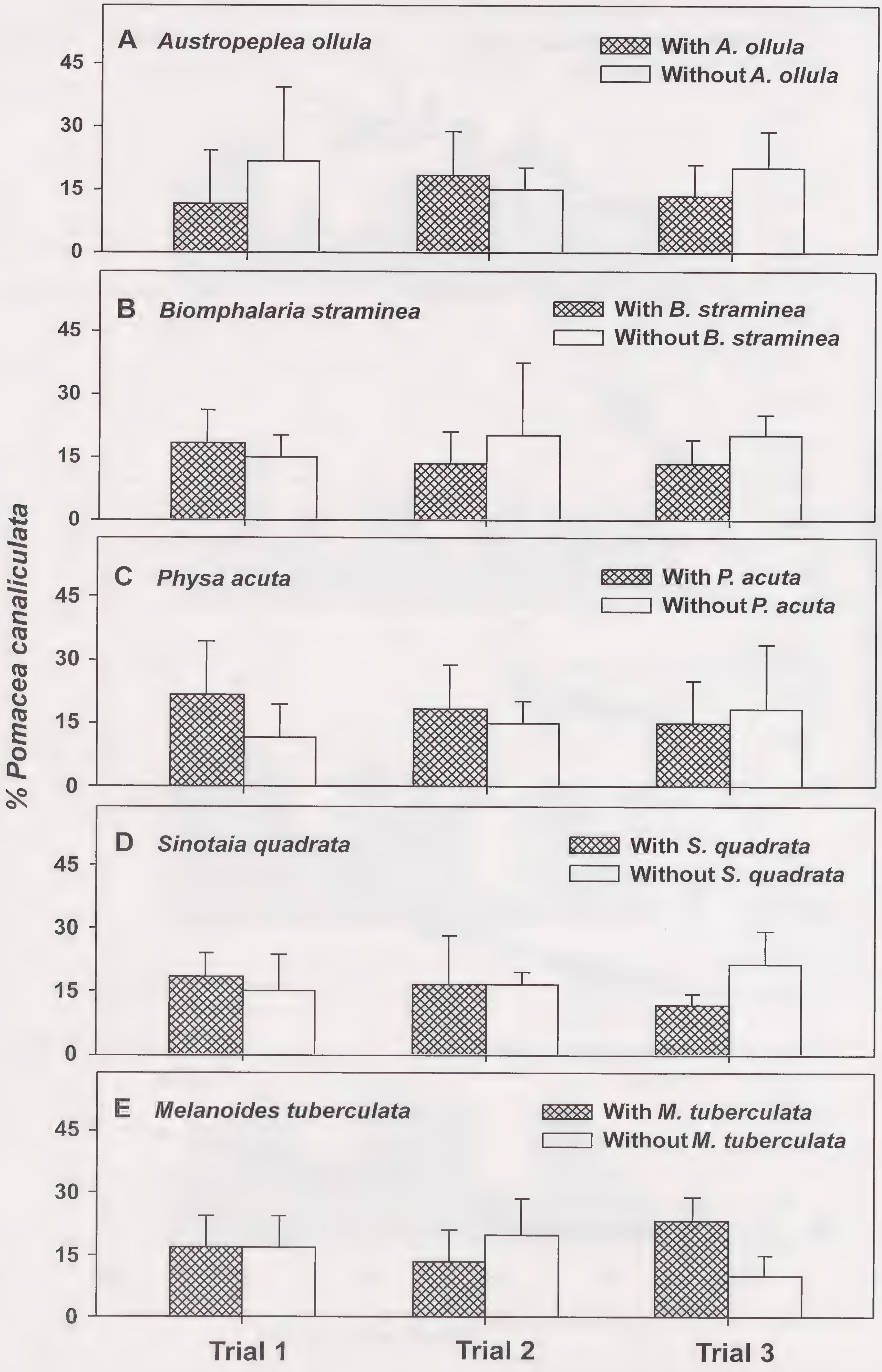

FIG. 5. Percentage of the predator in compartments containing the prey snail and those not containing the prey snail. Each species was tested in three separate assays. Each bar represents the mean of three replicate compartments ± S.D.

tuberculata were consumed, but the consumption was confined to ≤ 5 mm SL *S. quadrata* and ≤ 7 mm SL *M. tuberculata*; larger individuals were unaffected (Fig. 3).

Experiment 3. Crushing Resistance of Prey Species

For each species, the crushing resistance increased with increasing size (Fig. 4). In *A. ollula*, *B. straminea* and *M. tuberculata*, the relationship between shell size and crushing resistance could be described by a simple linear relationship. In *P. acuta* and *S. quadrata*, the relationship was exponential. The maximum crushing resistance in the three pulmonates was < 1 kg, which was equivalent to the crushing resistance of a much smaller prosobranch (6.6 mm SL *S. quadrata* or 7.6 mm SL *M. tuberculata*). The maximum crushing resistance of *S. quadrata* and *M. tuberculata* was 7.2 kg and 8.9 kg, respectively. The minimum shell length above which potential prey were safe from predation, from Experiment 2, corresponded to a crushing resistance of 0.75 kg for *S. quadrata* and 0.85 kg for *M. tuberculata*.

Experiment 4. Prey Detection and Crawling in *P. canaliculata*

The percentage of *P. canaliculata* located in the test and control compartments at the end of the experiment ranged from 10 to 23% (Fig. 5). For the five prey species, *t*-tests did not detect significant differences in the percentage of apple snails in the test and control compartments (*A. ollula*: $t = -1.11$, $P = 0.38$; *B. straminea*: $t = -1.00$, $P = 0.42$; *M. tuberculata*: $t = 0.38$, $P = 0.74$; *P. acuta*: $t = 0.87$, $P = 0.48$; *S. quadrata*: $t = -0.56$, $P = 0.64$).

In the crawling assays, the snail extended its foot fully and crawled away from the centre of the tray. Upon reaching the edge, it sometimes crawled up the wall briefly and returned to the bottom. Most of the time, it crawled on the bottom near the walls, with occasional movements toward the center. During the 1-h assay, the snail might make one or four stops. The longest stop was four minutes. Including these stops, average speed was 17.8 ± 1.7 cm min^{-1} ($n = 9$).

DISCUSSION

Using pre-starved *Pomacea canaliculata*, Cha (1989) reported predation on the egg masses and neonates of *B. straminea*, as well as the neonates of *A. ollula*, *P. acuta*, and *M. tuberculata*, but not on the neonates of *S. quadrata*. In our study, non-starved *P. canaliculata* fed on the neonates of all these species, although the consumption rate on *S. quadrata* was much lower than that on other species; they also consumed the adults of the three pulmonates. These results indicate that *Pomacea canaliculata* is a generalist predator of freshwater snails belonging to different families. Several other ampullariids are known to prey on various life stages of other snails. For example, *Pomacea bridgesii* ingested the eggs of *Indoplanorbis exustus* (Planorbidae) (Aditya & Raut, 2002), *Lanistes varicus* consumed the eggs and neonates of *Biomphalaria pfeifferri* (Planorbidae) (Anto et al., 2005), and *Marisa cornuarietis* preyed on the eggs, juveniles and adults of *Bulinus truncatus* (Planorbidae) (Demian & Lutfy, 1965a), juveniles and adults of *Lymnaea caillaudi* (Lymnaeidae) (Demian & Lutfy, 1966), eggs and neonates of *Biomphalaria glabrata* (Cedeño-León & Thomas, 1983), and eggs and neonates of *Biomphalaria alexandrina*, but not its adults (Demian & Lutfy, 1965b). Most of these prey species studied, however, belong to the Lymnaeidae and Planorbidae, which are intermediate hosts of human parasites, especially *Schistosoma*. The only practical way of combating these parasitic diseases is to control their vector snails (Dillon, 2000). Biological control using predators is traditionally conceived as environmentally friendly because it does not require the use of toxic chemicals, it is usually cheaper and its effects are long-lasting (Cowie, 2001). In fact, a number of field studies have demonstrated the usefulness of some ampullariids in controlling the populations of these vectors (reviews by Dillon, 2000; Cowie, 2001).

Due to the emphasis on human health, these previous studies have focused on the efficacy of predation, that is, whether the vector snails can be removed. Consequently, little information is available on the impact of these ampullariids on non-target snails (Cowie, 2002). Although anecdotal observations have linked the invasion of *P. canaliculata* to the decline of *Pila* spp. (Ampullariidae) in southeast Asia (Acosta & Pullin, 1991; Halwart, 1994), Cha (1989), our study is the first to provide experimental evidence of its relatively non-selective predation on several life-stages of various freshwater gastropod species, some of which are important detritivores in freshwater wetlands (Dudgeon & Yipp, 1985). The predation rates varied with the life stage of the predator, and the species and life stage of the prey. But in general, these rates were high, with daily consumption of an adult apple snail ranging from 21.0 (*A. ollula*) to 26.2 (*P. acuta*) egg masses, 1.5 (*S. quadrata*) to 5.2 (*P. acuta*) neonates, and 0.0 (*M. tuberculata* and *S. quadrata*) to 0.6 (*A. ollula*) adults. Cazzaniga (1990) reported an adult *P. canaliculata* killing only 0.067 ± 0.014 adult *Biomphalaria peregrina* per day, perhaps due to the large shell of the prey (9–13 mm). An adult *Pomacea bridgesii* consumed only around three egg masses of *Indoplanorbis exustus* per day, probably due to the lower predatory potential or smaller size of *P. bridgesii* compared to *P. canaliculata* (Aditya & Raut, 2002). High consumption rates have been reported in studies of other ampullariids. For example, Anto et al. (2005) reported that an adult *Lanistes varicus* ate up to 200 eggs masses and 1.8 neonates of *Biomphalaria pfeifferi* per day. Cedeño-León & Thomas (1983) reported that an adult *Marisa cornuarietis* devoured up to 50 egg masses of *Biomphalaria glabrata* per day.

Availability of macrophytic food may affect predation by ampullariids, but evidence as to whether it will enhance or reduce predation is inconsistent. For instance, Cedeño-León & Thomas (1983) reported that juvenile (17–23 mm) *Marisa cornuarietis* did not consume *Biomphalaria glabrata* eggs in the absence of macrophytic food. But once the macrophyte supply was resumed, it fed with a rate of 1.9 egg masses per snail per day. This primarily macrophytophagous snail may therefore need a minimum amount of macrophytic food to maintain its vigour for intensive browsing and/or predation. Nevertheless, Demian & Lutfy (1966) showed that an adult *Marisa cornuarietis* consumed only 0.29 adult *Bulinus truncatus* per day when fed with excess lettuce, but the consumption rate rose to 0.68 individuals per day in the absence of lettuce. Whether the relatively high predation rates in our experiments are due to the presence of lettuce is unknown, but they are lower than those reported by Cha (1989), who conducted the experiments with pre-starved *P. canaliculata* adults and found consumption rates of up to 180 egg masses and 31 neonates of *B. straminea* per day per individual.

To understand the impact of an invasive species on an invaded ecosystem, it is essential to collect empirical evidence of its interaction with indigenous species. But from a predictive perspective, it is perhaps more important to know how the invasive species affects indigenous species. *Pomacea canaliculata* can detect the presence of macrophytic food (i.e., *Elodea canadensis*, *Myriophyllum elatinoides*, *Rorippa nasturtium aquaticum* and *Zannichellia palustris*) from a distance of 30 cm (Estebenet, 1995). Romaine lettuce can also effectively attract *P. canaliculata* from a distance of 20 cm (Kwong, personal observation). Nevertheless, this study did not indicate that *P. canaliculata* could detect its prey from a distance. It appeared to crawl randomly in the aquaria. But direct contact of its tentacles with the prey did occasionally trigger an attack. The question thus remained as to whether such random crawling would create sufficient encounters between the predator and the prey. Dudgeon & Lam (1985) studied the crawling of seven species of freshwater gastropods in Hong Kong, four of which were used in this study. The mean crawling speed of *Biomphalaria straminea*, *Melanoides tuberculata*, *Physa acuta*, and *Sinotaia quadrata* was 3.73, 2.09, 6.76 and 2.25 cm min^{-1}, respectively. These rates of movement were far below that of *P. canaliculata* (17.8 cm min^{-1}). Based on a conservative 8-h active period per day, assuming the snail is only active at night, an adult foot width of approximately 2.5 cm, and a crawling speed of 17.8 cm min^{-1}, we estimate that the crawling of an adult *P. canaliculata* would cover an area of 2.1 m^2. Therefore, although this species cannot detect, or at least is not attracted to the water-borne cues released from other freshwater snails, it may still pose a threat to other snails due to its rapid crawling behavior.

The five prey species tested in this study differed greatly in their vulnerability to predation by *P. canaliculata*. The pulmonates appeared more vulnerable than the prosobranchs. Such differential vulnerability may be explained by the effectiveness of their defence. In general, a snail responds to a threat of predation in one of three ways: withdrawal of its head-foot into the shell, clinging tightly onto the substratum, or shaking its shell (reviewed by Dillon, 2000). The adaptive nature of the first two responses is obvious, but that of the latter may be best explained by the work of Townsend & McCarthy (1980), who studied the responses of *Physa fontinalis* upon direct contact with the tissue of molluscivorous leeches. The prey snail shakes its shell vigorously, followed by detachment of its foot from the substratum. Depending on whether this shaking results in enclosing an air bubble from the environment or spilling an air bubble held by the snail, the snail can float to the surface or sink to the bottom, thus escaping predation.

These three types of responses were all observed in this study. The two species of prosobranchs responded to contact with

Pomacea canaliculata by simply withdrawing their head-foot, pulling their operculum behind to close the shell. Sometimes *P. canaliculata* was seen to wrap the prey with its foot, and appeared to move its mouth and radula on the shell. Apparently due to the large size, hard shell and an operculum in these prey, the attacks always failed. After a brief encounter, the predator would move away. The three pulmonates responded differently. *Biomphalaria straminea* reacted initially by clinging onto the substratum. The predator might simply move the foot and body over the prey, leaving the *B. straminea* unharmed. Sometimes the predator twisted its foot, thus dislodging the prey from the substratum. The prey then retracted its head-foot. Occasionally the predator would initiate an attack by attempting to introduce its head into the shell aperture. Probably due to the small size of the prey and its fragile shell, the defence behaviours of *B. straminea* appeared ineffective. The shell aperture was usually broken. Due to the small aperture of the shell, some tissue usually remained uneaten. Other types of shell damage, as reported for *B. peregrina* (Cazzaniga, 1990), were only occasionally seen. Adult *Physa acuta* responded by shell-shaking. *Austropeplea ollula* held firmly onto the substratum and lowered its shell to minimize exposure. Both species have a large aperture but lack an operculum and thus are vulnerable when turned upside down. However, these two pulmonates tend to float on the air-water surface, which could reduce the chance of encounter with the predator. Sometimes an attack would be initiated if the predator was able to move its foot on top of the prey. The attack was similar to that on *Bulinus truncatus* by *M. cornuarietis* (Demian & Lutfy, 1965a). The predator would use its foot to orient the shell of the prey such that it could push its head into the shell aperture and use its radula and jaws to snip off the soft body of the prey. The predator would sometimes pull out the whole soft body. This attack behaviour therefore usually left an intact but empty shell.

Our experiments were conducted in the laboratory so no refuges were available. In the natural environment, plants, stones, and sediment might reduce the efficiency of predation. Also, in temperate and subtropical areas, *P. canaliculata* suffers high mortality and appears to aestivate during the winter (Cowie, 2002; Estebenet & Martín, 2002), which might allow the juveniles of some winter-breeding species to reach a size refuge and thus escape predation by *P. canaliculata*. In fact, *Sinotaia quadrata* is often found to co-exist with apple snails in lowland water bodies in the New Territories, Hong Kong. It carries fully developed hatchlings throughout the year, and birth mostly occurs in the warmer months (Dudgeon & Corlett, 2004), although we have seen small numbers of neonates in the winter (Kwong, unpublished data). Nevertheless, given its high crawling speed and relatively non-discriminating attacking behaviour, the possible predatory effect of *P. canaliculata* on freshwater snails, especially those with a fragile shell, should not be overlooked. In a 2 m cage study in an outdoor pond, introducing *P. canaliculata* caused a remarkable reduction in the density of six out of eight species of freshwater snails (Wong et al., in press). Taken together, the results of these field and laboratory studies indicate that predation by *P. canaliculata* may have a high potential for reducing the diversity of freshwater snails. This study provides further evidence supporting the precautionary principle when considering this and other ampullariids as biological control agents of snail vector of human parasites or aquatic weeds (Cowie, 2001).

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MOLLUSC BIODIVERSITY AND ENDEMISM IN THE POTENTIAL ANCIENT LAKE TRICHONIS, GREECE

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ABSTRACT

Ancient lakes are hotspots of biodiversity, often harboring a large number of endemic species that make them prime model systems for evolutionary biologists. Besides such well-recognized ancient or long-lived lakes as Baikal, Biwa, Ohrid, and Tanganyika, there are other potentially old and biodiverse lakes in the world with poorly specified ages and under-studied faunas. We here report on the mollusc fauna of one such lake, Lake Trichonis in continental Greece. This graben lake is situated in a highly tectonized area, characterized by karst features and probably of middle to late Pliocene origin. Lake Trichonis is deep, oligotrophic, and rich in such specific habitat types as macrophyte meadows, rocky shores and sublacustrine spring systems. Moreover, it is a hotspot of freshwater biodiversity in Greece, particularly in molluscs.

After reviewing newly collected material and the published mollusc records, we found that at least 33 mollusc species occur in Lake Trichonis, with 24 gastropod and 9 bivalve species currently being recognized. This is 24% of the total freshwater mollusc diversity of Greece; 21% of the gastropods (five species) are endemic to Lake Trichonis. If the whole Trichonis Basin is considered, which also includes neighboring Lake Lysimachia, eight species (33%) of the total fauna appear to be endemic. Taking lake surface areas into account, the index of gastropod endemism of 0.442 ($\log N_{\text{endemic species}}/\log A_{\text{surface area}}$) for the Lake Trichonis Basin resembles on a world-wide scale values known for Lake Baikal, Russia, and Lake Biwa, Japan, and is only exceeded by Lake Ohrid, Macedonia/Albania, and ancient lakes of Sulawesi, Indonesia.

Despite the limited knowledge about the lake's evolutionary history, the suggested age of origin, the palaeogeographical characteristics, and the potential timing of phylogenetic events reviewed here support the presumed status of Lake Trichonis as an ancient lake.

From a conservational standpoint, more research, management and conservation efforts are necessary because ancient lakes are among the most vulnerable and threatened ecosystems on earth. Effects of human-induced environmental change are already noticeable in Lake Trichonis. Recognition of Lake Trichonis as a unique system with an unusually high biodiversity may help promoting conservation efforts.

Key words: Ancient lake, Balkan, diversity, endemism, conservation.

INTRODUCTION

Ancient lakes are commonly hotspots of biodiversity, often harboring remarkable radiations that make them important targets for study by evolutionary biologists (e.g., Brooks, 1950; Martens et al., 1994; Martens, 1997; Rossiter & Kawanabe, 2000). Although ancient lakes may have different origins and characteristics, it is the diversity and endemism of their organisms that separate them from short-lived, post-glacial lakes. The latter parameters often serve as proxies for the recognition of such lakes (Martens, 1997). However, longevity at least since before the last glacial period (i.e., ~120,000 years ago) or even since before the Pleistocene some 1.8 Million years ago (Mya) (Wilke et al., 2008; Frank Riedel, personal communication) is often considered to be the only objective criterion for the recognition of ancient lakes (Gorthner, 1994; Martens, 1997). Unfortunately, often the age of a given lake is poorly constrained, resulting in confusion about its designation as an ancient lake. Moreover, a lake may have come into existence a long time ago but may not have existed continuously

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(e.g., Lake Victoria; Seehausen, 2006), thus not qualifying as ancient lake.

This and restricted knowledge of the faunas partly account for the existence of a number of unrecognized ancient lakes in the world that are outshone by their famous counterparts. Both the Balkan Peninsula and Asia Minor possess such candidate lakes, which all show some degree of endemism (e.g., Albrecht et al., 2006a; Wilke et al., 2007; Albrecht & Wilke, 2008). In the Balkans, there is the outstanding and well-known ancient Lake Ohrid, which has been in focus of evolutionary biology research for more than 100 years (review: Albrecht & Wilke, 2008). Less recognized are Lake Prespa (Crivelli & Catsadorakis, 1997) and almost all other Balkan lakes, such as Lake Skutari (Reed et al., 2004; Glöer & Pešić, 2008a), Lake Dojran (Griffith et al., 2002), and Lake Pamvotis (Frogley & Preece, 2004, 2007). Most of these lakes share a relatively high degree of endemic biodiversity, particularly in gastropods. However, they also have in common poorly understood systematics of the resident species. Taxonomic uncertainty (Rintelen et al., 2007) or the presence of cryptic species (Gomez et al., 2002; see also Wilke & Falniowski, 2001) can lead to an underestimation of the actual degree of diversity and endemism. To infer evolutionary processes in lacustrine systems, to understand ecological interactions, and to make informed conservation decisions, a robust taxonomic framework is required.

A candidate ancient lake for such studies is the western Greek Lake Trichonis (the old Greek name), which is also called Lake Trichonida (the new Greek name), or Lake Vrakhori (from the medieval name for the city of Agrinio located northwest of the lake). We use here the old name, since it is predominant in the current literature. The lake, situated in the South Adriatic-Ionian biogeographic region (Bănărescu, 2004), is the largest and deepest natural lake in Greece (Tafas et al., 1997).

The biodiversity and endemism of some of its faunal elements, particularly endemic molluscs, have received increasing attention in the past years (e.g., Reischütz & Reischütz, 2003; Szarowska et al., 2005; Albrecht et al., 2007; Glöer et al., 2007; Hauswald et al., 2008; Radea et al., 2008). Yet information is still sparse and partly controversial, due to the absence of a comprehensive faunal assessment for the lake in particular and for Greek freshwater molluscs in general. Only recently, has an attempt been made to summarize present knowledge (Bank, 2006). A principal problem in the study of Balkan freshwater molluscs is the adoption of a central European taxonomic framework. Thus, distinct taxa in this southwestern European region often remain unrecognized.

During ongoing studies of the evolution and diversity of molluscs in European ancient lakes (e.g., Albrecht et al., 2006a, b; Albrecht & Wilke, 2008), we surveyed most large natural lakes on the Balkans, among them Lake Trichonis. During this field work and a survey of the published mollusc record for the lake, we realized that Lake Trichonis, in terms of mollusc biodiversity and endemism, is among the most outstanding but largely ignored lakes in the Balkans.

We therefore attempt in the present review to (a) re-evaluate the lake's mollusc fauna, (b) to compare its molluscan biodiversity and endemism on a Balkan and a global scale, (c) to discuss the potential "ancient" character of Lake Trichonis, and (d) assess the conservation status of the lake's mollusc fauna.

GEOLOGY AND LIMNOLOGY OF LAKE TRICHONIS

Lake Trichonis (Fig. 1) has been classified as a warm, monomictic and karstic water body (Zacharias et al., 2002), embedded within a complex hydrogeological system (Tafas et al., 1997a, b; Zacharias et al., 2003; Elias & Ierotheos, 2006), and situated in a highly tectonized area (Poulimenos & Doutsos, 1997). Many different rock formations account for the complex regional geology of the Trichonis Basin containing Lake Trichonis and neighboring Lake Lysimachia. The basin is bounded by a major north-dipping fault system on the south side (Goldsworthy et al., 2002). According to Zacharias et al. (2003), fissured limestone dominates in the catchment of the basin, whereas

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FIGS. 1–4. Lake Trichonis and selected characteristic habitat types. FIG. 1: View from North of the lake; FIG. 2: Oligotrophic conditions with hard substrates in the upper littoral followed by a typical *Chara* belt; FIG. 3: Rocky shore at the NE steep parts of Lake Trichonis with marks of different water levels visible at the rock's surface; FIG. 4: Lakes Trichonis and Lysimachia (Greece) and collecting points of the 2005 and 2007 surveys. Bathymetric lines according to Zacharias et al. (2003).

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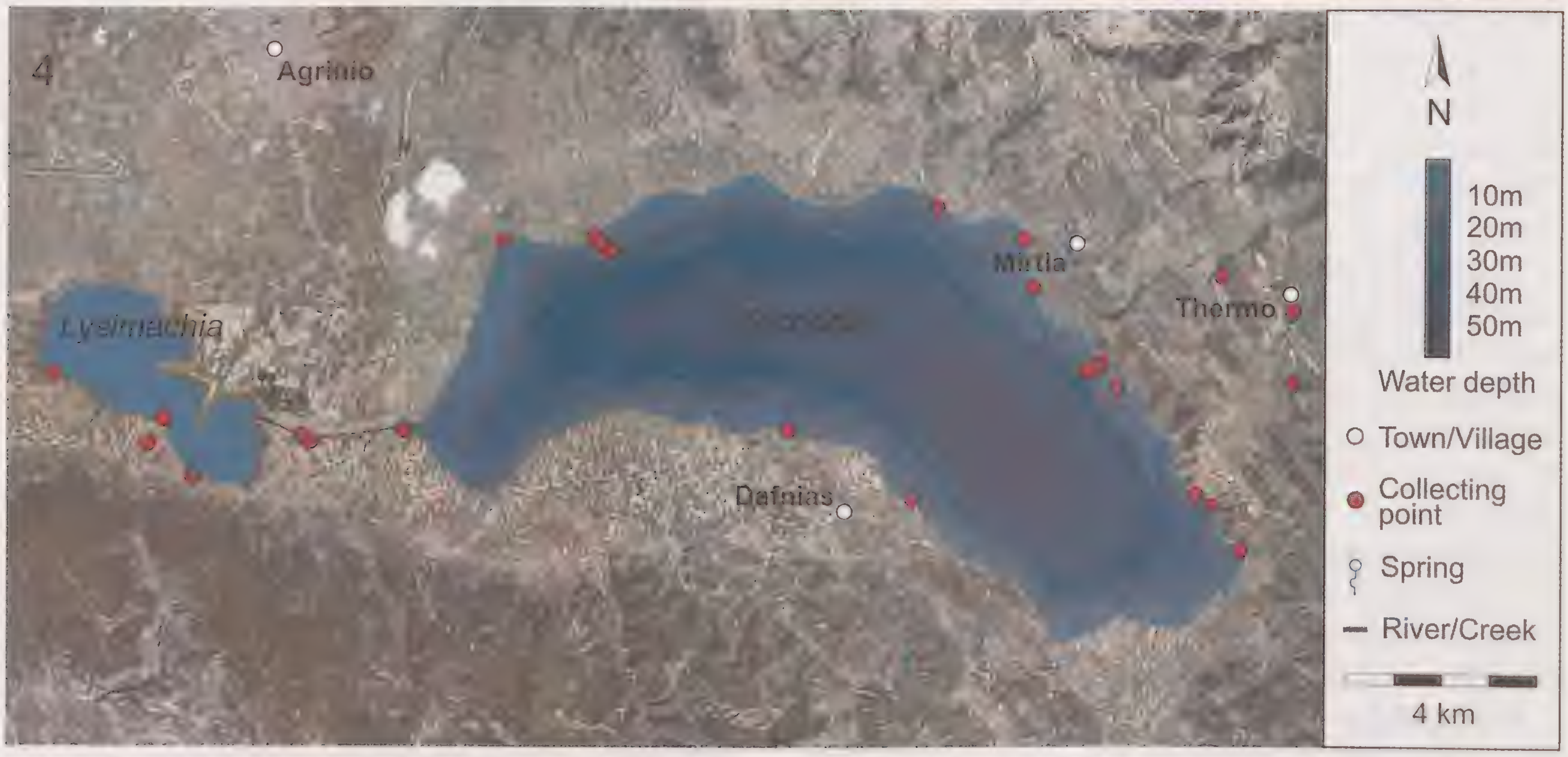
4
Agrinio
Lysimachia
Mirtia
Thermo
Dafnias
N
10m
20m
30m
40m
50m
Water depth
○ Town/Village
● Collecting point
Spring
River/Creek
4 km

TABLE 1. Selected characteristics of Lake Trichonis (*data from Tafas et al., 1997; **data from Zacharias et al., 2002).

	Lake Trichonis
Location	38.508°–38.603°N; 21.445°–21.657°E
Altitude	16 m a.s.l.
Maximum Length	17.8 km
Maximum Width	6.5 km
Maximum Depth	58 m*
Mean Depth	30.45 m*
Volume	2.93 km^3**
Shore length	52.07 km
Surface area	94.24 km^2
Retention time	9.4 years**
Secchi depth (min/max)	8.5/13.0 m**
Catchment area	421 km^2**
Annual water level fluctuation	1 m*
Climate type	Csa (Koeppen)

fault uplifts folded Miocene flysch deposits and Eocene limestones in its footwall, with some young lacustrine sediments occurring in the western part of the footwall, which steps to the north (Goldsworthy et al., 2002).

With a maximum depth of 58 m and a surface area of 92.4 km^2, Lake Trichonis is the deepest and largest natural lake in Greece (Table 1, Figs. 1–4). Preferential groundwater flows are enhanced by karstic conditions characteristic for the area. Faulting processes are most pronounced in the northeast of the lake (Zacharias et al., 2003).

Chemically, Lake Trichonis is considered as a carbonate type, low conductivity lake, with relatively low nitrogen and phosphorous concentrations (Tafas et al., 1997; Tafas & Economou-Amilli, 1997).

Today's water balance of Lake Trichonis is characterized by inflows from approximately 30 seasonal streams, direct precipitation on the lake surface, and subaquatic karstic springs (Elias & Ierotheos, 2006). It is considered to be an oligotrophic water body, with a maximum Secchi disk depth of 13 m and a residence time of 9.4 years (Zacharias et al., 2002). Output occurs via a connection to neighboring Lake Lysimachia and from there via the River Acheloos into the Adriatic Sea (Overbeck et al., 1982).

Annual lake level changes of approximately one meter are caused by the lake's hydraulic relation with karst aquifers, by evaporation during the hot, dry summer, and by intensive irrigation and water extraction (Tafas et al., 1997; Tafas & Economou-Amilli, 1997; Fig. 3). Water extraction increased considerably when a hydraulic infrastructure was constructed in 1957 (Elias & Ierotheos, 2006).

The littoral of the lake is characterized by a variety of habitats, including large zones of macrophytes, as well as rocky and sandy shores (Figs. 1–3). The vertical (bathymetrical) zonation of major habitat types in the lake shows the same differentiation described for other ancient Balkan lakes, such as Lake Ohrid (Radoman, 1985; Albrecht & Wilke, 2008): a rocky or sandy upper littoral (Fig. 2), the *Chara*-belt, a shell zone with both dead and live *Dreissena* specimens, a sandy and silty zone without shells, and the profundal. However, compared to Lake Ohrid, there is a bathymetric shift of these zones towards shallower water depths. Whereas, for example, the shell zone in Lake Ohrid extends to 35 m (Albrecht & Wilke, 2008), in Lake Trichonis it typically only extends to 20 m (Wilke et al., unpublished data).

These geological and limnological features of Lake Trichonis – an likely old basin combined with a relatively large size and considerable depth, as well as a diverse watershed (including springs and temporal streams), and a clear horizontal and vertical zonation of the lake proper – have contributed to a relatively high degree of freshwater biodiversity and endemism in the lake, particularly in molluscs.

MOLLUSC BIODIVERSITY AND ENDEMISM

Mollusc Biodiversity

For Lake Trichonis, a total of 45 extant mollusc taxa is mentioned in the literature, of which we consider at least 33 species (24 gastropod and 9 bivalve species) to occur in the lake today (Table 2). The difference of 12 species is due to both changed taxonomy and nomenclature, but also decline and potential loss, as well as recognition of new species. All taxa with authorships are listed in Table 2. For the entire freshwater fauna of Greece, Bank (2006) listed a total of 112 gastropod and 25 bivalve species (and subspecies). Thus, Lake Trichonis is home to 21% of the Greek freshwater gastropod diversity and 36% of the bivalve diversity (24% of total freshwater mollusc fauna).

Two species of the gastropod genus *Pseudobithynia* – *P. falniowskii*, *P. panetolis*, – as

TABLE 2. Comparative species list and type of endemism of mollucs occurring in Lake Trichonis. Literature records: S – Schütt (1962, 1980), RR – Reischütz & Reischütz (2003), R – Radoman (1983, 1985); FS – Dyduch-Falniowska (1989), Falniowski & Szarowska (1995), Falniowski (1999), Falniowski et al. (1988, 1997), Szarowska et al. (2005); RLE – Radea et al. (2008); KPT – Koussouris & Pugh-Thomas (1982); ASWHW – Albrecht & Schultheiß (September 2005) and Wilke, Hauffe, Wolff (September 2007). Nomenclature largely follows Bank (2006). Presence/validity: Critical evaluation of the published record and new field data. ()species for which no recent record exists. Levels of endemicity: $E_{\text{Trichonis}}$ – endemic to Lake Trichonis; $E_{\text{TriLysOzPam}}$ - endemic to Lakes Trichonis and/or Lysimachia and/or Ozeros and/or Pamvotis; E_{Greece} – endemic to continental Greece (Bank, 2006), i.e. the southern Balkans.

Species	S	R	RR	FS	KPT	RLE	ASWHW	Presence/ validity	Scale of endemism
Neritimorpha									
Theodoxus varius callosus (Deshayes, 1835)			x				x	x	E_{Greece}
Theodoxus danubialis (C. Pfeiffer, 1828)	x^1				x^1				
Caenogastropoda									
Viviparus ater hellenicus (Clessin, 1879)	x		x	x	x		x	x	E_{Greece}
Viviparus cf. *viviparus* (Linnaeus, 1758)			x^2		x^2				
Bithynia graeca (Westerlund ,1879)			x^3						
Bithynia majewskyi Frauenfeld, 1862	x^4								
Pseudobithynia falniowskii (Glöer & Pešić, 2006)							x	x	$E_{\text{Trichonis}}$
Pseudobithynia sp.							x	x	$E_{\text{Trichonis}}$
Pseudobithynia trichonis (Glöer, Albrecht & Wilke, 2007)							x	x	E_{TriLys}
Pseudobithynia panetolis (Glöer, Albrecht & Wilke, 2007)							x	x	$E_{\text{Trichonis}}$
Potamopyrgus antipodarum J. E. Gray, 1843						x		x	
Trichonia trichonica Radoman, 1973		x						(x)	E_{TriPam}
Pseudoislamia balcanica Radoman, 1979		x^5						x	$E_{\text{Trichonis}}$
Islamia trichoniana Radoman, 1979		x	x					(x)	$E_{\text{Trichonis}}$
Belgrandiella haesitans (Westerlund, 1881)	x							x	E_{Greece}
Dianella thiesseana (Clessin, 1878)	x	x	s	x			x	x	E_{TriLys}

(continues)

(continued)

Species	S	R	RR	FS	KPT	RLE	ASWHW	Presence/ validity	Scale of endemism
Heterobranchia									
Valvata cristata O. F. Müller, 1774	x		x		x		s	x	
Valvata cf. *macrostoma* Mörch, 1864			s						
Valvata piscinalis (O. F. Müller, 1774)	x		s	x	x		s	x	
Valvata klemmi Schütt, 1962	x		s		x		x	x	$E_{TriLysOz}$
Acroloxus lacustris (Linnaeus, 1758)	x		x					x	
Galba truncatula (O. F. Müller, 1774)			x				x	x	
Stagnicola cf. *fuscus* (C. Pfeiffer, 1821)			s						
Radix auricularia (Linnaeus, 1758)			x[6]						
Radix balthica (Linnaeus, 1758)				x[7]					
Radix labiata (Rossmässler, 1835)					x[8]				
Radix sp.							x	x	E_{TriLys}
Haitia acuta (Draparnaud, 1805)			x				x	x	
Planorbarius corneus (Linnaeus, 1758)			x					x	
Ferrissia wautieri (Mirolli, 1960)			s				x	x	
Planorbis carinatus O. F. Müller, 1774	x		s		x			x	
Planorbis planorbis (Linnaeus, 1758)			x				x	x	
Gyraulus laevis (Alder, 1838)	x[9]				x[9]				
Gyraulus sp. juv.			x						
Hippeutis complanatus (Linnaeus, 1758)	x		s				s	x	

(continues)

(continued)

Species	S	R	RR	FS	KPT	RLE	ASWHW	Presence/ validity	Scale of endemism
Bivalvia								x	
Unio crassus ionicus Drouët, 1879			x				s	x	E_{Greece}
Pisidium amnicum (O. F. Müller, 1774)			x	x				x	
Pisidium casertanum (Poli, 1791)				x				x	
Pisidium personatum Malm, 1855			x					x	
Pisidium nitidum Jenyns, 1832			x	x				x	
Pisidium subtruncatum Malm, 1855				x				x	
Pisidium tenuilineatum Stelfox, 1918				x				x	
Pisidium annandalei Prashad, 1925				x				x	
Dreissena blanci Westerlund, 1890	x		x				x	x	
Dreissena polymorpha (Pallas, 1771)					x[10]				

[1] *T. danubialis* is not known to occur in Greece (Bank, 2006) and is most likely mistaken by the authors.

[2] Cited as "*V. fasciatus* Mull." which appears to be usually a synonym of *Viviparus viviparus*. This species, however, is restricted to the lowlands N of the Alps. It most likely does not occur in Lake Trichonis.

[3] Since no other recent study found *B. graeca* in Lake Trichonis (Glöer & Pešić, 2006) we consider this to be likely a *Pseudobithynia* sp.

[4] Species from Croatia not recognized for Greek fauna (Bank, 2006).

[5] Spelled as *balkanica* by Radoman (1983: 83); see Bank (2006) for the taxonomy of that species.

[6] *Radix auricularia* not found during recent collections at Lake Trichonis, likely belonging to *Radix* sp.

[7] *Radix balthica* not found during recent collections in the southern Balkans, maybe belonging to *Radix* sp.

[8] Cited as *Radix peregra* f. *ovata*, most likely *Radix* sp.

[9] Considering Meier-Brook (1983: 38), it is unlikely that *Gyraulus laevis* occurs in the Mediterranean region.

[10] Today regarded as *Dreissena blanci* (see Albrecht et al., 2007).

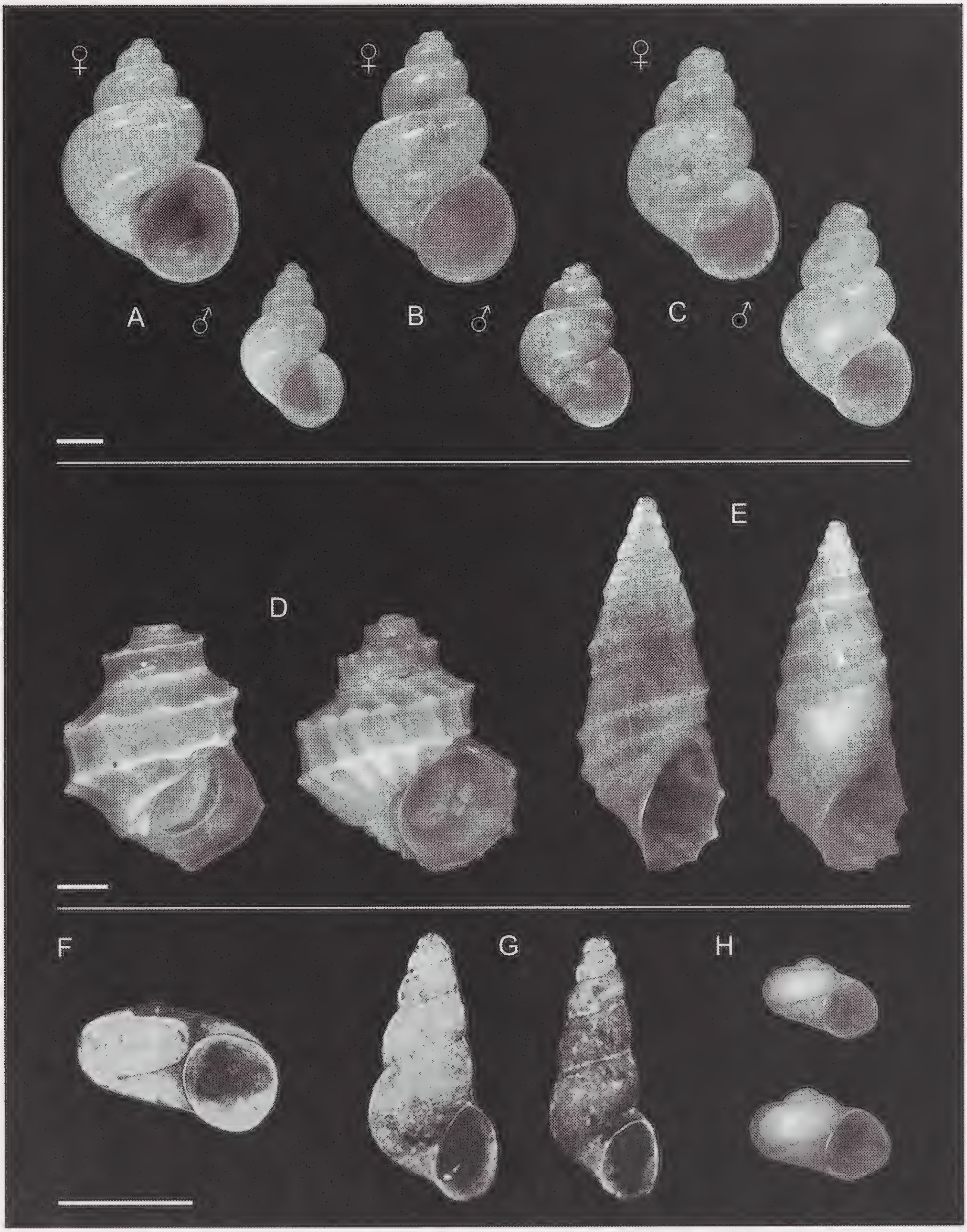

FIG. 5. Endemic gastropod species of Lake Trichonis. A: *Pseudobithynia falniowskii*; B: *Pseudobithynia panetolis*; C: *Pseudobithynia trichonis*; D: *Valvata klemmi*; E: *Dianella thiesseana*; F: *Islamia trichoniana*; G: *Trichonia trichonica*; H: *Pseudoislamia balcanica* - juvenile on top, subadult on bottom. Images of the bithyniid species are taken from original descriptions of Glöer & Pešić (2006) and Glöer et al. (2007). *Trichonia trichonica* and *Islamia trichoniana* reproduced from Radoman (1983; Plate V, No. 82, 83, page 220; Plate IX, No. 153, page 228). Scale bar = 1 mm.

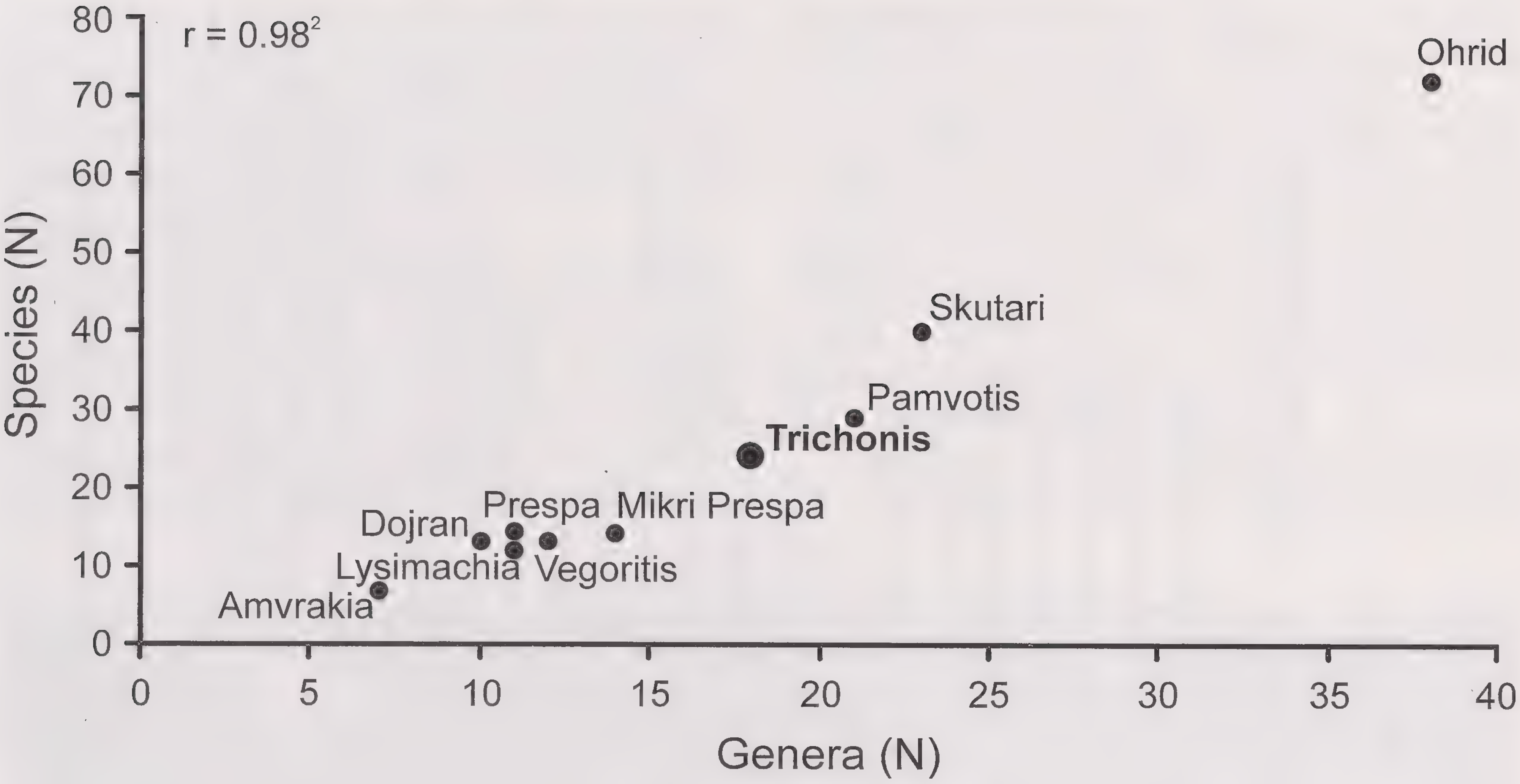

FIG. 6. Correlation between species and generic diversity in selected recognized and potential ancient lakes on the Balkan Peninsula.

well as *Pseudoislamia balcanica* and *Islamia trichoniana* are considered to be endemics to Lake Trichonis (Fig. 5). In addition, two taxa are considered to be new for science and endemic to the lake: *Radix* sp. and *Pseudobithynia* sp. (Glöer, personal communication).

Dianella thiesseana and *Pseudobithynia trichonis* only occur in Lake Trichonis and neighboring Lake Lysimachia. *Valvata klemmi* is now known from lakes Trichonis, Lysimachia and Ozeros (Wilke et al., unpublished data). *Trichonia trichonica*, a species previously found exclusively at its type locality in Lake Trichonis, was recently found at Lake Pamvotis (Frogley & Preece, 2007). The remaining known faunal elements from Lake Trichonis are either subspecies considered to be regionally distributed (i.e., *Theodoxus varius callosus*, *Viviparus ater hellenicus*, and *Unio crassus ionicus*) or widespread species.

Lake Trichonis has a greater number of both gastropod species and genera compared to many other Balkan lakes, such as Prespa. However, Lake Trichonis has less taxonomic diversity than lakes Pamvotis, Skutari or Ohrid (Fig. 6). It should be noted that the intensity of faunistic studies has varied greatly among those lakes. Thus, numbers presented here for other Balkan lakes should be regarded as preliminary.

In the case of Lake Trichonis, it is not unlikely that new collections will reveal new mollusc species. For example, the bithyniid *Pseudobithynia falniowskii* was formerly subsumed under *Bithynia graeca* and only recently recognized as new and endemic for Lake Trichonis (Glöer & Pešić, 2006). In fact, almost none of the assumed widespread taxa of Lake Trichonis have yet been studied in a detailed phylogenetic context, with the possible exception of *Dreissena* "*polymorpha*" (Table 2; see also Albrecht et al., 2007). The phenomenon of undetected diversity is not restricted to molluscs or invertebrates in this system. Even the already diverse and well-studied fish fauna of Lake Trichonis was recently enlarged by a new species of blenny, *Salaria economidisi* Kottelat, 2004 (Wirtz, 2006).

More molluscan endemics are likely to be discovered in Greek lakes. Only such detailed studies as those of Frogley & Preece (2004, 2007) in Lake Pamvotis may eventually close the gaps in knowledge.

Gastropod Endemism

Endemism in freshwater habitats is subject to intense studies (e.g., Dudgeon et al., 2006) and, as endemism can occur at different spatial scales, especially narrow-range taxa have been of interest to researchers (e.g., Ponder & Colgan, 2002).

At the lake scale, 21% of the gastropods (five out of 24 species) are endemic to Lake Trichonis. At the scale of the Trichonis Basin, 33% (eight species) of the total fauna appear to be endemic.

TABLE 3. Gastropod endemism in selected Balkan and world-wide ancient lakes compared to Lake Trichonis.

Lake	Total N species	N endemic species	Endemism (%)	Surface area (km^2)	Index [$\log N_{\text{endemic species}}/\log A_{\text{surface area}}$]	Source of endemism data
Worldwide ancient lakes						
Baikal	147	114	78	31,500	0.457	Strong et al. (2008)
Biwa	38	19	50	674	0.452	Strong et al. (2008)
Malawi	28	16	57	29,600	0.269	Rintelen et al. (2007)
Malili lakes	61	58	95	753	0.613	Rintelen et al. (2007)
Poso	25	23	92	323	0.543	Rintelen et al. (2007)
Tanganyika	73	66	90	32,600	0.403	West et al. (2004)
Titicaca	24	15	63	8448	0.300	Dejoux & Iltis (1992)
Balkan lakes						
Amvrakia	7	2	29	14.2	0.261	Albrecht et al. (2006a)
Dojran	13	1	8	42.8	0.000	Stankovic (1985)
Lysimachia	12	0 (3)[1]	0 (25)[1]	13.5	0.000 (0.422)[1]	Reischütz & Reischütz (2003)
Mikri Prespa	14	2	14	53	0.175	Radoman (1985), Albrecht & Wilke unpublished data
Trichonis	24	5 (8)[2]	21 (33)[2]	96.5	0.352 (0.442)[2]	this study
Ohrid	72	56	78	358	0.685	Albrecht & Wilke (2008)
Pamvotis	29	2 (5)[3]	7 (17)[3]	22	0.224 (0.521)[3]	Frogley & Preece (2004, 2007)
Prespa	14	6	43	266	0.321	Radoman (1985), Albrecht & Wilke unpublished data
Skutari	40	7	18	600[4]	0.304	Glöer & Pešić (2008a, b), Glöer in litt.
Vegoritis	13	2	15	53	0.175	Schütt (1962, 1985), Albrecht et al. (2006a), Albrecht & Wilke unpublished data

[1] Number in brackets if sister Lake Trichonis is considered.

[2] Number in brackets if sister Lake Lysimachia is considered.

[3] Data for Lake Pamvotis are equivocal with the estimated minimum endemism and maximum endemism including taxa of unclear status (Frogley & Preece, 2007).

[4] Average maximum surface area.

Given the tentative value of 33% gastropod endemism, Lake Trichonis Basin is far exceeded by the famous Balkan lakes Ohrid (78%) and Prespa (43%). However, it is considerable higher than other Balkan lakes, such as Lake Skutari (18%) (Table 3).

On a global scale, gastropod endemism in Lake Trichonis does not reach the levels of > 50% found in such ancient lakes as lakes Baikal, Poso, Tanganyika, Titicaca, or the Malili lakes (Table 3). However, compared to these lakes, Lake Trichonis is relatively small. Thus, for comparative reasons, and applying island biogeographic theory to freshwater gastropod biodiversity (e.g., Lassen, 1975), the respective area (e.g., lake surface or watershed) may be incorporated into calculation of endemic biodiversity indices. Taking lake sizes into account, a double log value of 0.442 of number of endemic species per lake surface areas results for the Lake Trichonis Basin. With this relatively high value, Lake Trichonis is second on the Balkan scale and even far exceeds such famous lakes as Malawi and Titicaca (Table 3).

In addition to geographical scale, it might be informative to assess the taxonomical ex-

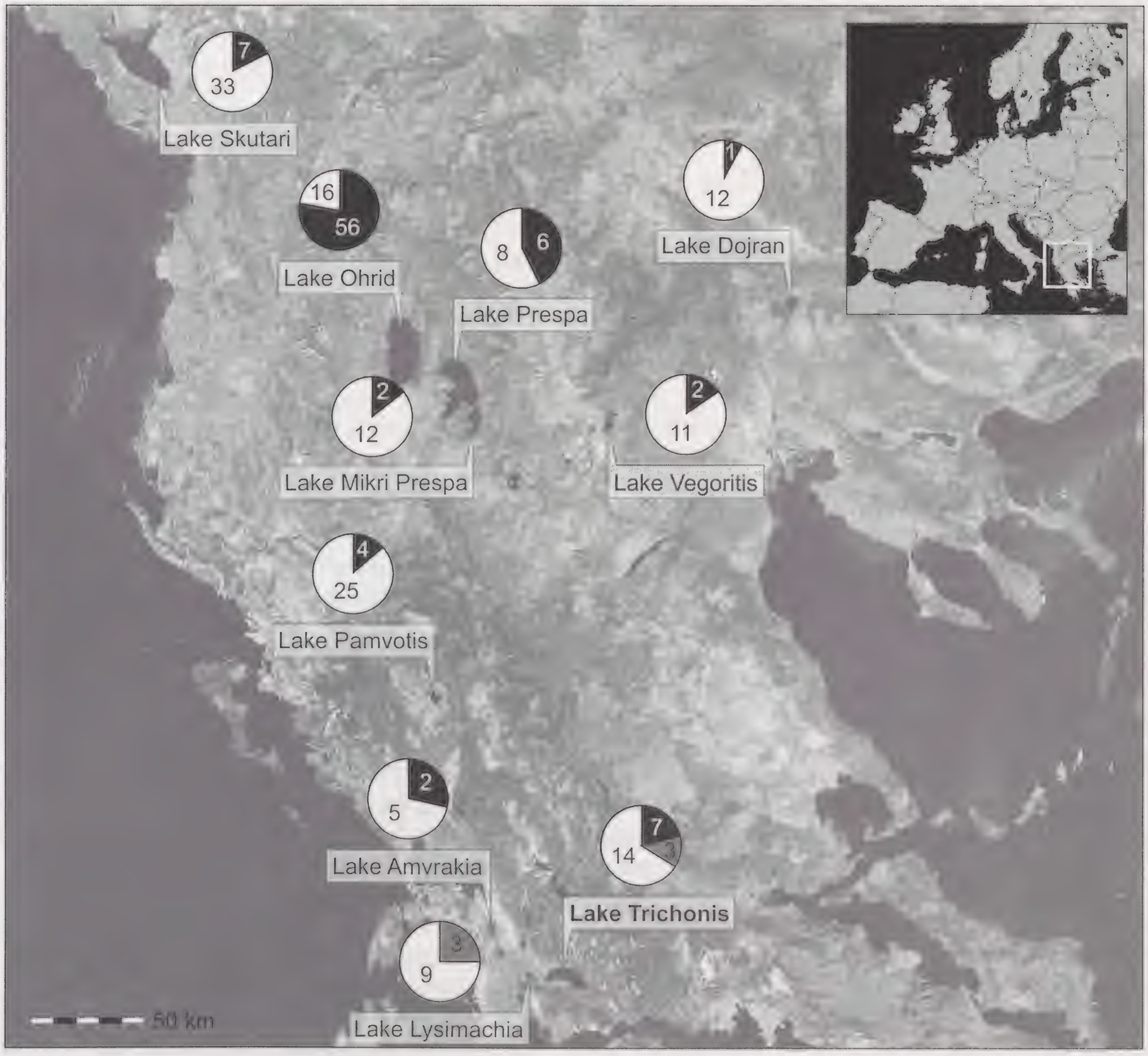

FIG. 7. Gastropod endemism in selected recognized and potential ancient lakes on the Balkan Peninsula. Black slices indicate percentage of endemic species (references see Table 3), total numbers are plotted. Data for Lake Pamvotis represent the maximum endemism including taxa of unclear status (Frogley & Preece, 2007). In Lake Trichonis, black indicates strict endemics while grey accounts for species also occurring in the sister Lake Lysimachia (see Table 1). Lake Lysimachia shares 3 endemics with its sister Lake Trichonis.

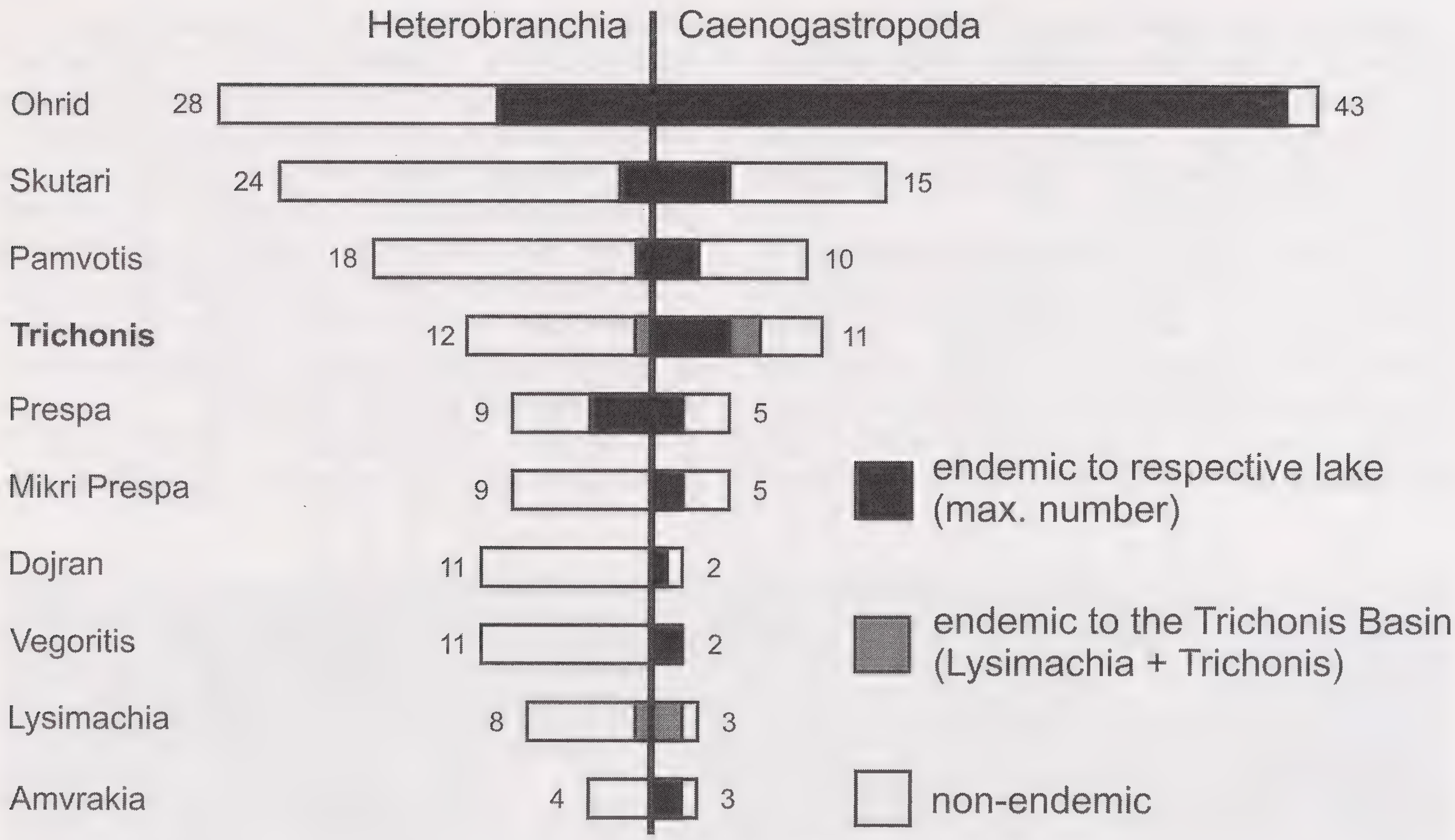

FIG. 8. Gastropod taxonomic composition in selected recognized and potential ancient lakes on the Balkan Peninsula. Note that this statistics is restricted to the two major clades Caenogastropoda and Heterobranchia but does not include *Theodoxus* spp. Data for Lake Pamvotis are maximum estimates of endemism including taxa of unclear status (Frogley & Preece, 2007). In Lake Trichonis, black indicates strict endemics while grey accounts for species also occurring in the sister Lake Lysimachia (see Table 1).

tent of endemism, that is, whether endemism occurs at the supraspecific level. Such well-recognized ancient lakes as Baikal or Ohrid are characterized by endemic genera or even family-level taxa.

Lake Trichonis harbors the endemic and monotypic hydrobiid genus *Pseudoislamia* Radoman, 1978. Beside lakes Ohrid and Trichonis, there are only two other Balkan lakes, Prespa and Mikri Prespa, that currently have an endemic genus each. Endemicity at the generic level, however, is difficult to assess in the absence of thorough phylogenetic and systematic analyses of a given biogeographical region.

Nonetheless, endemism at and above the species level in Lake Trichonis hints at a high degree of mollusc diversification both for the lake itself as well as for the Trichonis Basin (Figs. 4, 7).

Although eutrophic Lake Lysimachia and oligotrophic Lake Trichonis appear very different in their current limnological characteristics (Overbeck et al., 1982), they are sister lakes due their close proximity and direct hydrological connection, which is also evident in a fair degree of shared faunistic elements (for a discussion of ancient sister lakes, see Albrecht et al., 2008b).

A striking difference in the mollusc fauna composition of Lake Trichonis compared to lakes Ohrid, Tanganyika or the lakes on Sulawesi is the absence of species flocks, that is, monophyletic and endemic groups of species. However, there are other recognized ancient lakes in the world that also lack gastropod radiations. It remains to be studied whether the four endemic species of *Pseudobithynia* (Glöer & Pešić, 2006; Glöer et al., 2007) in Lake Trichonis represent a small species-flock.

In addition to elevated endemism, another common feature among ancient lake gastropod species is a higher shell sculpture index compared to ubiquitous species (Gorthner, 1992). A keel, for example, is a prominent shell feature not only of *Dianella thiesseana* from Lake Trichonis, but also of many pyrgulinid gastropod taxa, such as *Chilopyrgula sturanyi*, *Ohridopyrgula macedonica* and *Prespopyrgula prespensis* from the Balkan lakes Ohrid and Prespa, or *Falsipyrgula* from Lake Egirdir in Asia Minor (Wilke et al., 2007). *Valvata klemmi*

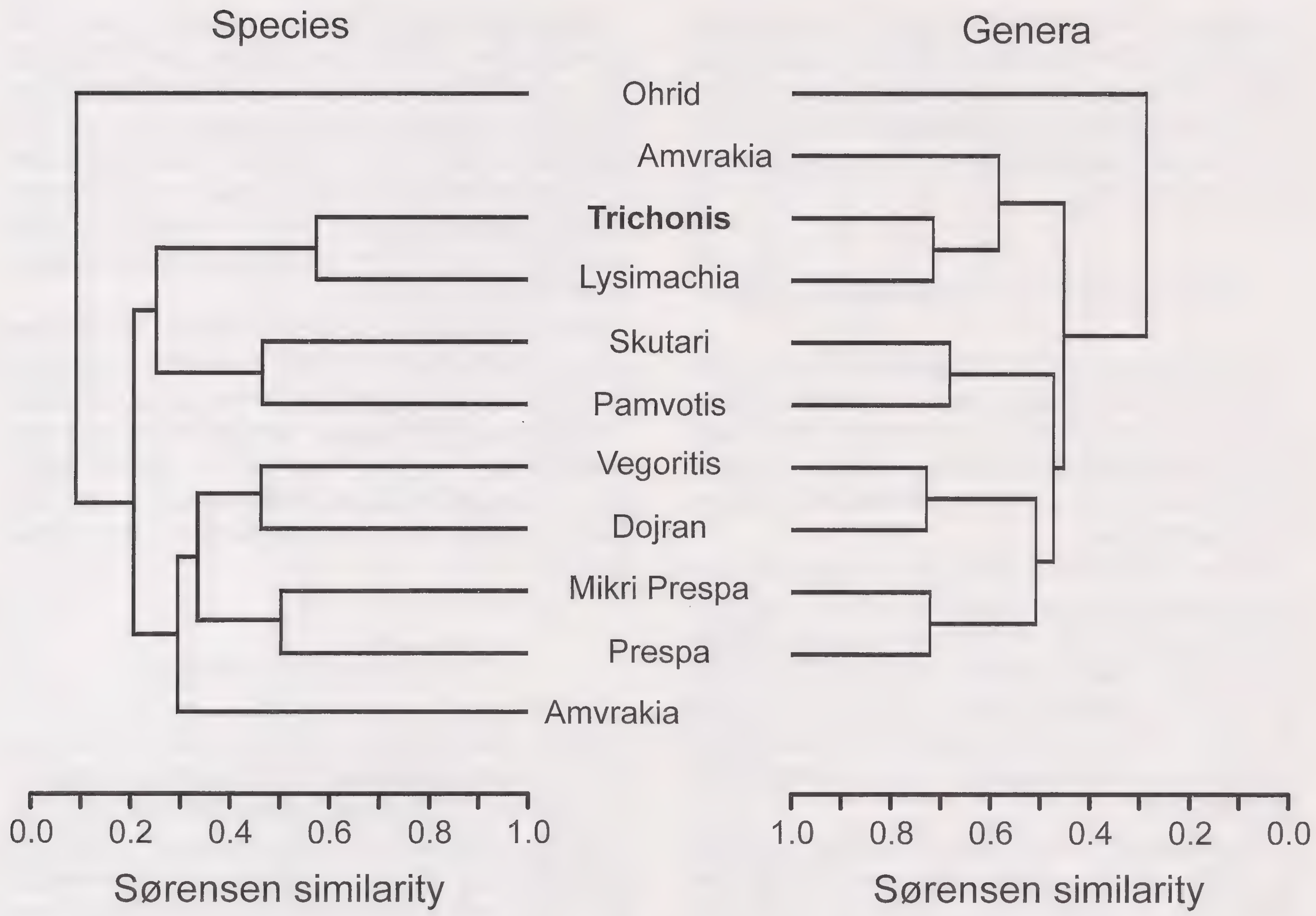

FIG. 9. Cluster analysis of faunal relationships at species (N = 147) and genus level (N = 61). The paired-group method and Sørensen-similarity were used and the analyses conducted in PAST 1.83 (Hammer et al., 2001).

is another Trichonis species with ornate shell sculpture resembling congeners endemic to Lake Ohrid (Fig. 5). A recent molecular study, however, raised doubts concerning the species status of that taxon (Hauswald et al., 2008).

The question whether certain gastropod clades are more prone to speciation in ancient lakes has been treated repeatedly (reviewed in Michel, 1994). Comparing the two major clades, that is, Caenogastropoda and Heterobranchia, the general trend of higher percentage endemism in the Caenogastropoda becomes evident in the Balkan lakes. Lake Trichonis is no exception (Fig. 8). With the exception of Lake Ohrid, Lake Trichonis ranks well with the other Balkan lakes Pamvotis, Skutari, and Prespa. The endemism of mainly pulmonates, however, is significantly higher in the latter.

Lake Trichonis endemism is not restricted to molluscs but is also evident in fishes. The well-studied fish fauna (Leonardos, 2004) holds interesting species, including the endemic *Economidichthys trichonis* (Gobiidae), the smallest known freshwater fish species of Europe (Economidis & Miller, 1990; Miller, 1990). Endemism also occurs in phytoplankton and periphyton (e.g., Kristiansen, 1980; Economou-Amilli, 1982; Falniowski et al., 1988; Economou-Amilli & Tafas, 2000).

Given the still scarce data on the living world of Lake Trichonis, the currently recognized degree of endemism in different taxa is remarkable. Future detailed studies of, for example, macrozoobenthic taxa would certainly give new insights into endemism, biogeography, and evolutionary history of Lake Trichonis.

Faunal Relationships among Balkan Lakes

The faunal relationships of the Lake Trichonis malacofauna was analyzed at species and generic levels (Fig. 9). Both analyses support the sister lake relationship to Lake Lysimachia. Based on presence/absence of genera, Lake Amvrakia is the most closely related lake to those sister lakes. In the species-level analysis, lakes Trichonis and Lysimachia clustered as sister group to lakes Skutari and Pamvotis.

Radoman (1985) classified Lake Trichonis as belonging to the so-called Adriatic-Ionian lake group. His analysis was based on species of the “Hydrobioidea” only and did not include all lakes considered here. Direct comparisons are thus not possible.

LAKE TRICHONIS AS ANCIENT LAKE

As mentioned above, longevity at least since before the last glacial period (ancient lakes *sensu lato*) or even since before the Pleistocene (ancient lakes *sensu stricto*) is often considered to be the only objective criterion for ancient lakes.

Given recent progress in studies of the palaeogeographical evolution of the Balkans (Steininger & Rögl, 1984; Popov et al., 2004), hydrological data available for Lake Trichonis, as well as existing dated phylogenetic data for some of its endemic taxa, it is possible to comment on the status of Lake Trichonis as ancient lake.

Unfortunately, there are no detailed geological and/or limnological data available for the exact age of extant Lake Trichonis. However, Economidis & Miller (1990) hypothesized the tectonic origin of the lake in the Late Pliocene. Khondkarian et al. (2004) suggested that the Trichonis Basin could have been formed in the southernmost edge of a freshwater/lowland area east of the Hellenids in the Middle to Late Pliocene (i.e., some 3.4 to 1.8 Mya).

The oligotrophic and relatively deep Lake Trichonis is situated in the Trichonis Graben in a highly tectonized area with Karst limnology. These characteristics: oligotrophic status, which often is associated with low sedimentation rates, large original depth, and tectonic origin are also typical characteristics of other ancient lakes on the Balkans (e.g., Lake Ohrid; Albrecht & Wilke, 2008). These conditions allow for a compensation of sedimentation by subduction. In addition, Karst conditions assure water input during periods of low precipitation and thus might prevent desiccation.

Recent molecular clock analyses suggested the split of *Dianella thiesseana* (subfamily Pyrgulinae) from Lake Trichonis and its sister taxon, the Lake Ohrid pyrgulinid species flock, to be between 2.6 ± 0.3 My (Wilke et al., 2009) and 2.8 ± 0.3 My old (Wilke et al., 2007). Moreover, Trichonis populations of the fish genus *Leuciscus* are estimated to have separated from river populations 2.2 Mya (Doadrio & Carmona, 1998).

Although timing of these events cannot be used as direct evidence for the ancient character of Lake Trichonis, they hint at Plio-Pleistocene divergence events, possibly associated with the origin of Lake Trichonis and at the distinctness of some of its taxa. In fact, Doadrio & Carmona (1998) even consider Lake Trichonis (including River Evinos) to form a biogeographical subregion within Greece.

Although detailed paleostratigraphic studies remain to be done on Lake Trichonis, biological information both from taxonomic uniqueness of the fauna and molecular divergence of selected taxa indicate a Plio-Pleistocene age or older. Thus, it qualifies as an ancient lake *sensu lato*, and possibly even as an ancient lake *sensu strictu*.

CONSERVATION ISSUES

Many ancient lakes in the world are famous for their extraordinarily high degree of mollusc endemism. However, these faunas are frequently under extreme anthropogenic pressure (Coulter et al., 2006). Therefore, more research, management and conservation efforts are necessary because ancient lakes are among the most vulnerable and threatened ecosystems (Lévêque et al., 2005).

The endemic mollusc species of many of the Balkan lakes were described only a few decades ago. The decline and potential loss of endemic mollusc diversity in lakes in Montenegro, Albania, Macedonia and Greece has been described by Bouchet et al. (1999). The urgency of conservation measures was highlighted by Albrecht et al. (2006a) as lakes in circum-Mediterranean countries are under ever increasing anthropogenic pressure. Major causes are dramatic changes in water levels due to massive extraction for agribusiness. At the same time, the eutrophication-level of most lakes increased during the past decades due to a combination of water use, fishery, pollution, toxification, and climatic extremes, especially during summer. These factors lead to direct or indirect habitat destruction and loss. As a consequence, communities change and eventually complete food webs become disrupted. Highly adapted and specialized species cannot cope with these environmental changes, which can happen very quickly in some cases, for example, during a single season.

From a conservation point of view, it is necessary to assess the current status of the endemic species of Lake Trichonis. Reischütz

TABLE 4. Ecology and conservation measures of endemic gastropods of Lake Trichonis. This table includes described endemic species only. Distribution and habitat characteristics are taken from Radoman (1983), Schütt (1962, 1980) but also include own observations.

Species	Distribution	Habitat	Depth range	Conservation status
Trichonia trichonica Radoman, 1973	NE shore near Mirtia (Radoman, 1983), S coast (Schütt, 1980)	rocky bank	sublittoral (Schütt, 1980)	no recent records
Pseudoislamia balcanica Radoman, 1979	NE shore near Mirtia	rocky bank	1 m	critically endangered
Islamia trichoniana Radoman, 1979	NE shore near Mirtia	stony	unknown	no recent records
Pseudobithynia falniowskii (Glöer & Pešić, 2006)	Southern shore	gravel, *Dreissena* beds, including macrophytes	5 m	endangered
Pseudobithynia trichonis (Glöer, Albrecht & Wilke, 2007)	NE shore	stones	1 m	endangered
Pseudobithynia panetolis (Glöer, Albrecht & Wilke, 2007)	E of Panetolion	rocks	1 m	endangered
Dianella thiesseana (Clessin, 1878)	NE shore	gravel sand	10–27	critically endangered
Valvata klemmi Schütt, 1962	S shore and S shore of Lake Lysimachia (Schütt, 1962); NW and NE shore of Lake Trichonis and Lake Ozeros (this study)	stones, gravel, pebble, sand, mud	0–27	endangered

& Reischütz (2003) only found shells of *Dianella thiesseana*, whereas Szarowska et al. (2005) could collect very few specimens. During our survey in 2007 and 2008, we could find this taxon at only one sampled locality. Little over 20 years ago, Radoman (1985) reported that *D. thiesseana* was very common in Lake Trichonis, which is certainly not the case anymore. A similar situation is present for other hydrobiid endemics. *Trichonia trichonica* and *Islamia trichoniana,* as well as *Pseudoislamia balcanica*, all described from rocky or stony bank parts of the lake, were assumed to be extinct (Szarowska, 2006). We found only *Pseudoislamia balcanica* in very low abundances at two localities near Mirtia on the northeastern rocky shore of Lake Trichonis.

Effects of human-induced environmental changes are evident for Lake Trichonis (Bertahas et al., 2006), with water level loss and eutrophication (mostly due to increased phosphorous loads) being the most serious threats. These processes are particularly severe for microsnails living in a narrow stretch of the littoral (Table 4). Fast water level and/or biofilm changes have a great impact on these highly adapted communities.

Changes are recognizable in the whole ecosystem, for example, by the presence of invasive fish species (Leonardos et al., 2008). Invasive gastropod species become more and more important in ancient lakes, as outlined for *Physa acuta* in Lake Titicaca (Albrecht et al., 2008b), a species also present in Lake Trichonis. The very recent appearance of another globally invasive gastropod species, *Potamopyrgus antipodarum*, in Lake Trichonis is of even greater concern (Radea et al., 2008). These circumstances and the reported decline in endemic gastropod diversity, should trigger efforts to save this sensitive lake ecosystem. By 2008, none of the Trichonis lake endemic molluscs are listed in the IUCN Red List of Threatened Species.

A critical point in all conservation efforts related to Balkan lakes is public awareness of the uniqueness of these ecosystems, which may help increasing the acceptance of, for example, management plans for controlled water extraction. Sewage treatment systems should be installed along the actual lakes and all tributary systems. Agricultural practices should become more sustainable with reduction of fertilizer and pesticide use as ultimate goal. However, all these practices can only be effective if concerted action plans are implemented in the immediate future. A first step was achieved by listing the wetlands surrounding Lake Trichonis as Natura 2000 Environmental Protection Area.

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RESEARCH NOTES

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THE SEXOLOGY OF THE CHIRALLY DIMORPHIC SNAIL SPECIES *AMPHIDROMUS INVERSUS* (GASTROPODA: CAMAENIDAE)

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INTRODUCTION

Co-occurrence of both enantiomorphs of a chiral body form is found infrequently in nature, rather directional asymmetry (the presence of only one of the two possible enantiomorphs) is the rule (Palmer, 2004). Whereas directional asymmetry is normally genetic, the majority of the few dozen known cases of chiral dimorphism with equal proportions of the two enantiomorphs – "antisymmetry", as defined by Van Valen (1962) and Palmer (1996, 2005) – are non-inherited and randomized. So far, only two instances of genetic antisymmetry have been described, and both appear to involve reproductive benefits in their maintenance: flower morphology in *Heteranthera multiflora* and other enantiostylous plants, in which antisymmetry promotes outcrossing (Jesson & Barrett, 2002), and mouth orientation in the cichlid fish *Perissodus microlepis*, where disassortative mating exists (Takahashi & Hori, 2008; but see Van Dooren, unpublished data).

Conspicuous genetic chiral dimorphism is also found in snails of the Southeast Asian tree snail genus *Amphidromus* (Laidlaw & Solem, 1961; Asami et al., 1998; Craze et al., 2006; Schilthuizen & Davison, 2005; Schilthuizen et al., 2005, 2007; Sutcharit & Panha, 2006a, b; Sutcharit et al., 2006). Around 36 species are currently included in the subgenus *Amphidromus* s. str., of which at least 30 are chirally dimorphic (Schilthuizen et al., 2005). The other subgenus, *Syndromus*, consisting of approximately 50 species, is entirely sinistral; a species phylogenetically basal to these two clades, *A. glaucolarynx* (formerly placed in *Syndromus*) is, however, also chirally dimorphic, suggesting that chiral dimorphism is the ancestral condition for this genus (Sutcharit et al., 2006). In some species, proportions of dextral and sinistral snails do not deviate significantly from 50/50 proportions (e.g., in *A. martensi* Boettger, 1894; Craze et al., 2006), whereas in others, proportions can be dextral- or sinistral-biased (Sutcharit et al., 2006). However, proportions of both chiral morphs are almost always substantial, and though not strictly adhering to the definition of antisymmetry, we do not consider the patterns displayed by *Amphidromus* as essentially different from the other known cases of genetic antisymmetry referred to above.

In *Amphidromus inversus* (Müller, 1774), chiral dimorphism appears to enhance mutual fertility. Briefly, this species (and other *Amphidromus* s. str. species; Sutcharit & Panha, 2006b) has a coil in the spermatophore tail, which follows the coiling direction of the snail that produces it (dextral in a dextral individual, sinistral in a sinistral one). After copulation, the tip of this spermatophore coil rests in the recipient individual at the opening of the free oviduct. Since the free oviduct is connected to the spermatophore-receiving organ at an angle, the "fit" is better in dextral-sinistral (inter-chiral) pairs than in sinistral-sinistral or dextral-dextral (intra-chiral) pairs (for more details: Schilthuizen et al., 2007). Modelling (Schilthuizen et al., 2007; Craze, 2009) shows that this situation would, under panmixia, lead to equal proportions for dextral and sinistral morphs (true antisymmetry), but population structure will normally drive a bias for the recessive allele. To all intents and purposes, therefore, chiral dimorphism in *Amphidromus* should be considered a case of genetic antisymmetry.

The evidence for the maintenance of chiral dimorphism in *Amphidromus inversus* derives from studies in a population on the West-Malaysian island of Kapas. This population was discovered by one of us (M.S.) in 1989 and studied intensively since 2003 (Schilthuizen et al., 2005). Later, the same population was described as a new subspecies (*A. i. albulus*) by Sutcharit & Panha (2006a), but since the description is based only on shell colour and shell size, two characters known to be highly variable in *Amphidromus*, we refrain from following their suggestion.

We propose that *A. inversus* might be useful in the future for further studies of mollusc chiral-

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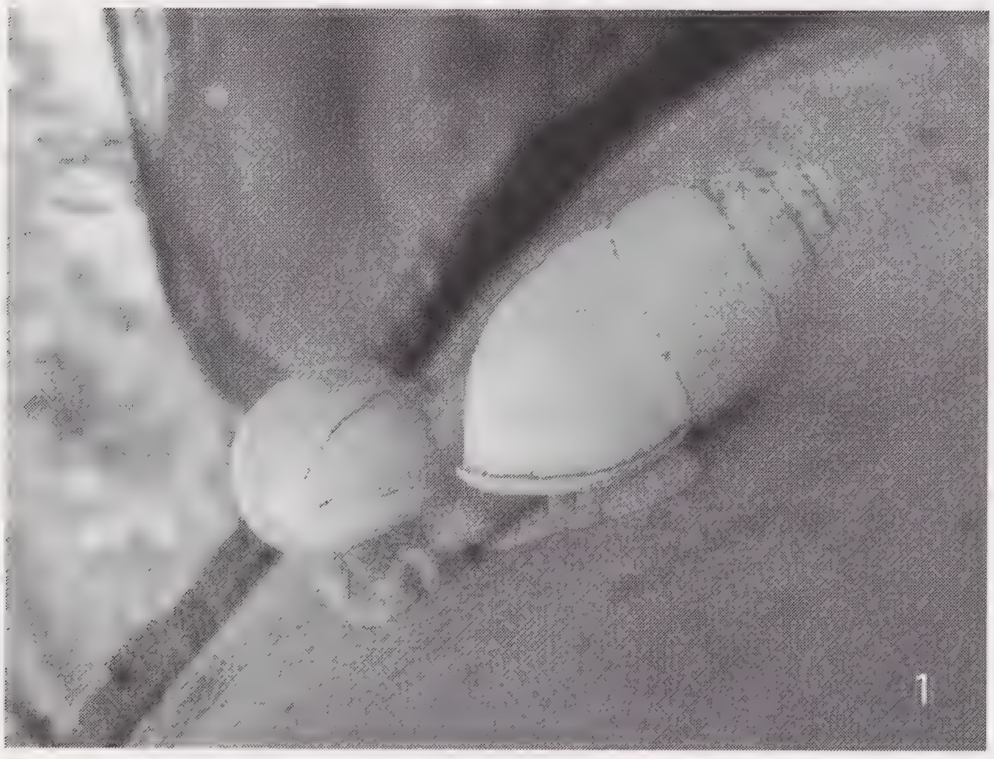
ity and antisymmetry, a field that is likely to become an important component of evolutionary developmental biology (Grande & Patel, 2009). We do so for the following reasons: (1) The population has high density and the snails are easily accessible on low trees in coastal forest. (2) The species' conchology and anatomy have been very carefully described and illustrated by Sutcharit & Panha (2006a, b). (3) Captive or semi-captive individuals are, have been, or will be in culture in the University of Nottingham (Angus Davison), the Edinburgh Zoo (Edwin Blake), the Randers Regnskov Tropical Zoo in Denmark (Asser Øllgaard), the Institute for Biology Leiden (Kees Koops), and in Malaysia (Menno Schilthuizen), and in some (but, admittedly, not all) of these settings, they have fared well, readily copulating, ovipositing and growing. To add to the basis of knowledge for this species, which may become more heavily used in mollusc chirality studies, in this paper we provide a description, based on field observations and field-collected animals only, of copulation and functional anatomy of the genitalia.

MATERIALS AND METHODS

We observed parts of the copulation, oviposition, and egg-hatching processes in the field on the West-Malaysian island of Kapas (5°13'00"N; 103°15'30"E; 1.7 × 0.7 km) during five field trips: 29 August–7 September 2003, 26–31 August 2004, 7–18 September 2005, 13–16 October 2005, and 18 May–6 July 2008. Observations were done between 7.00 and 17.00 h while searching snails randomly in coastal and primary forest on the island's west coast and in predefined research plots: sites 1 and 2 from Schilthuizen et al., 2007 (160 × 300 m and 240 × 70 m, respectively) and sites 3, 4, and 5 (previously unpublished, studied in 2008 only, each measuring 50 × 50 m). The positions of mating couples were recorded and, when possible, visited repeatedly throughout the day. We also searched at night on several occasions, roughly between 21.00 and 23.00 h. Twelve copulas in different stages of copulation progress, were fixed in the following way: the mating individuals (and in particular the parts of the genitalia that were extruded) were rapidly and thoroughly sprayed from all directions with electrical component freezing spray, as described in Schilthuizen & Lombaerts (1995) and Craze & Barr (2002), and, while thus frozen, immediately transferred to 70% ethanol. This way, it was assured that the internal genitalia were preserved as exactly as possible in the configuration that they had had during copulation, and the sequence of events during copulation could be reconstructed from them. One copula was carefully separated while in progress, which made both partners expel their spermatophores. The spermatophores were preserved in 70% ethanol. (Please note that we were limited by the copulas that were found in the field, and, though these represent different stages in the copulation process, they do not represent pre-set intervals or are in any way controlled observations.)

Copulas as well as 58 individual adult snails (collected and preserved while not in copula)



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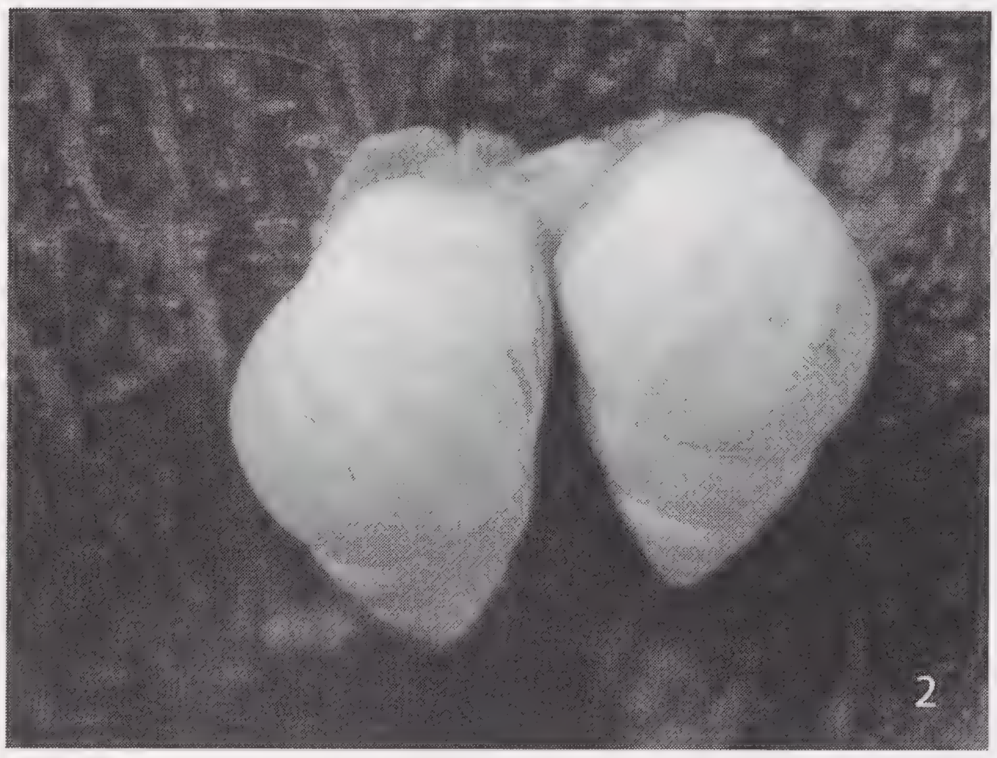

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FIGS. 1, 2. Copulating pairs of *Amphidromus inversus*, photographed in the field. FIG. 1: Conchologically mature S × S pair; FIG. 2: D × S pair in which at least the sinistral partner is conchologically immature.

were dissected by chipping away the shell(s) with a strong forceps and then dissecting away all non-reproductive organs. In some cases, the genitalia were cleared with clove oil. In all individuals, the bursa copulatrix was removed, opened, and checked for the presence of spermatophore fragments.

Of the egg clutches found in the field, five were hatched in the lab under dark, ambient conditions in bags made of nylon stocking. For the rest, the positions in the field were marked and they were regularly checked until hatching. In addition, three egg clutches were produced at the Edinburgh Zoo (Davison, pers. comm.). For 12 individuals from one of the field-collected egg clutches, total genomic DNA was isolated using a Qiagen DNA extraction kit. A cytochrome oxidase subunit I fragment (mtDNA, COI) was amplified using the universal COI primers of Folmer et al. (1994). Products were cleaned (Qiagen PCR purification kit) and directly sequenced on an ABI377 in both directions using an ABI BigDyePRISM kit.

RESULTS

Overall, we observed 92 copulating pairs (Fig. 1). Interestingly, some copulating individuals lacked a fully-formed apertural lip, indicating that sexual maturity precedes the end of shell formation (Fig. 2). Courtship is briefly described as follows: two individuals, upon meeting each other on a tree stem or branch, align their shells and then circle one another repeatedly. During this behavior, their heads touch now and then, and they mount each other's shells briefly and repeatedly. Individuals are also seen nibbling each other's palatal apertural shell walls. After approximately 30 minutes, either copulation is achieved or the partners reject one another and continue their separate ways. During the middle of the day (between noon and 15.00 h), mating couples appeared to be engaged in mid-coitus only, and the termination of mating was seen to occur only in the late afternoon and evening (between 15.00 h and 22.00 h). Hence, we estimate (with the caveat that our observation time was largely limited to daytime) that copulations typically last between 5 and 10 hours and are centred around mid-afternoon. Although conically-shelled snails such as *Amphidromus* generally mate non-reciprocally by shell mounting (Asami et al., 1998; Davison et al., 2005; Davison & Mordan, 2007), mating in *Amphidromus inversus* was reciprocal (this could be confirmed by inspecting the parts of the everted genitalia that were visible in between the copulating partners, and which always consisted of two penis-vagina complexes). Also, contrary to expectation, the snails did not engage in shell-mounting, but in variations on a face-to-face position, although the animals would keep their shells at angles of anything between zero to 180°, depending on their angle of approach and the inclination of the substratum. Some indication was found that the angle between the shells also depends on the coiling direction of the partners: for 39 mating pairs – 24 D × S, 9 S × S, 6 D × D – we measured the angle between the snails' columellae and found that almost all (12 out of 14) intra-chiral pairs held their shells at angles $> 45°$, whereas of all inter-chiral pairs, the angle in half (12 out of 25) was $< 45°$ (Fisher's exact test, $P < 0.04$). Although no systematic study was made, our field sketches suggest that inter-chiral pairs held the left side of the head of the sinistral partner against the right side of the head of the dextral partner, whereas inter-chiral copulas tended to copulate in a face-to-face manner, with the same side of the head touching in both partners. This is reflected in the difference in angles between their shells while in copula.

Despite these possible differences between intra- and inter-chiral pairs in body orientation during mating, the internal meshing of the genitalia appeared identical in both situations; since the genital organs are long, thin, flexible tubes, twisting to match the partners chirality appeared no great feat to accomplish. We dissected ten (out of 12 fixed) copulas, leaving their genitalia in situ. This revealed several consecutive stages in copulation. Both partners were always in almost exactly the same stage, hence, below, the actions of only one spermatophore "donor" and one spermatophore "recipient" are described. (We adopt the terminology used by Sutcharit & Panha, 2006b.)

Stage 1 (one copula; Fig. 3)

The everted penial verge of the donor is inserted into the recipient's vagina. The spermatophore is beginning to form inside the donor's epiphallus and flagellum; simultaneously, sperm is entering the epiphallus from the vas deferens.

Stage 2 (six copulas; Fig. 4)

The long spermatophore is fully formed, though still rather thin, and its body not yet fully filled with sperm. The epiphallus is much extended to accommodate it. The tail of the spermatophore remains in the donor's flagellum (its coiled expanded section [CES] remains fixed in the coiled part of the flagellum), and remains empty, while the front part of the spermatophore is being filled with sperm. At the same time, its tip is entering the vagina.

Stage 3 (two copulas; Fig. 5)

The tail of the spermatophore remains in the donor's flagellum (the coiled expanded section [CES] of the tail remains fixed in the coiled part of the flagellum), while the front part of the spermatophore is folded into the basal part of the recipient's gametolytic duct. The spermatophore's movement is accomplished by a shortening of the donor's epiphallus. Even as the spermatophore body is entering the gametolytic sac, more sperm continues to be pushed via the vas deferens into the part that is still in the epiphallus.

Stage 4 (one copula; Fig. 6)

The tail of the spermatophore is in the process of leaving the donor (the coiled expanded section [CES] of the tail is being pulled out of the coiled part of the flagellum), while the majority of the spermatophore is folded into the recipient's gametolytic duct.

Among the non-copulating individuals, we found no individuals with spermatophores in the male genitalia. This confirms that the spermatophore only begins to be formed upon initiation of copulation and not before. We did, however, find non-copulating individuals with entire or partial spermatophores in the gametolytic duct, which indicated that they had recently mated. This allowed us to recognise three more stages in the process of spermatophore transfer.

Stage 5 (two individuals, collected not in copula; Fig. 7)

The entire donor's spermatophore has been folded up into the recipient's gametolytic duct; the coiled expanded section of the spermatophore tail is jammed in the swollen basis of the gametolytic duct.

Stage 6 (one individual, collected not in copula; Fig. 8)

The entire donor's spermatophore is being fragmented in the recipient's gametolytic duct; the coiled expanded section of the spermatophore tail has moved up in the gametolytic duct and no longer closes the entrance to the gametolytic duct.

Stage 7 (six individuals, collected not in copula; Fig. 9)

The entire donor's spermatophore is fragmented and the fragments are being transferred to the gametolytic sac.

In all 78 preserved and dissected individuals (both copulating ones and non-copulating ones), we opened the gametolytic sac and in 28 individuals found recognisable fragments of spermatophores in the process of being digested (in cases where at the same time a spermatophore was present in the gametolytic duct, the already strongly-digested fragments in the sac were clearly from a much older copulation). Hence, the final eighth stage in the process of spermatophore transfer is:

Stage 8 (28 individuals, collected not in copula; Fig. 10)

The entire spermatophore has been fragmented and is being digested in the gametolytic sac.

We studied one dextral and one sinistral spermatophore. As described previously (Sutcharit & Panha, 2006b; Schilthuizen et al., 2007), the spermatophore is approximately 6 cm long, has five longitudinal, slightly crenulated ridges, and the part that is formed in the epiphallus has a central lumen that is filled with sperm (Fig. 4). The coiled expanded section (CES), which is formed in the tip of the flagellum, contains no

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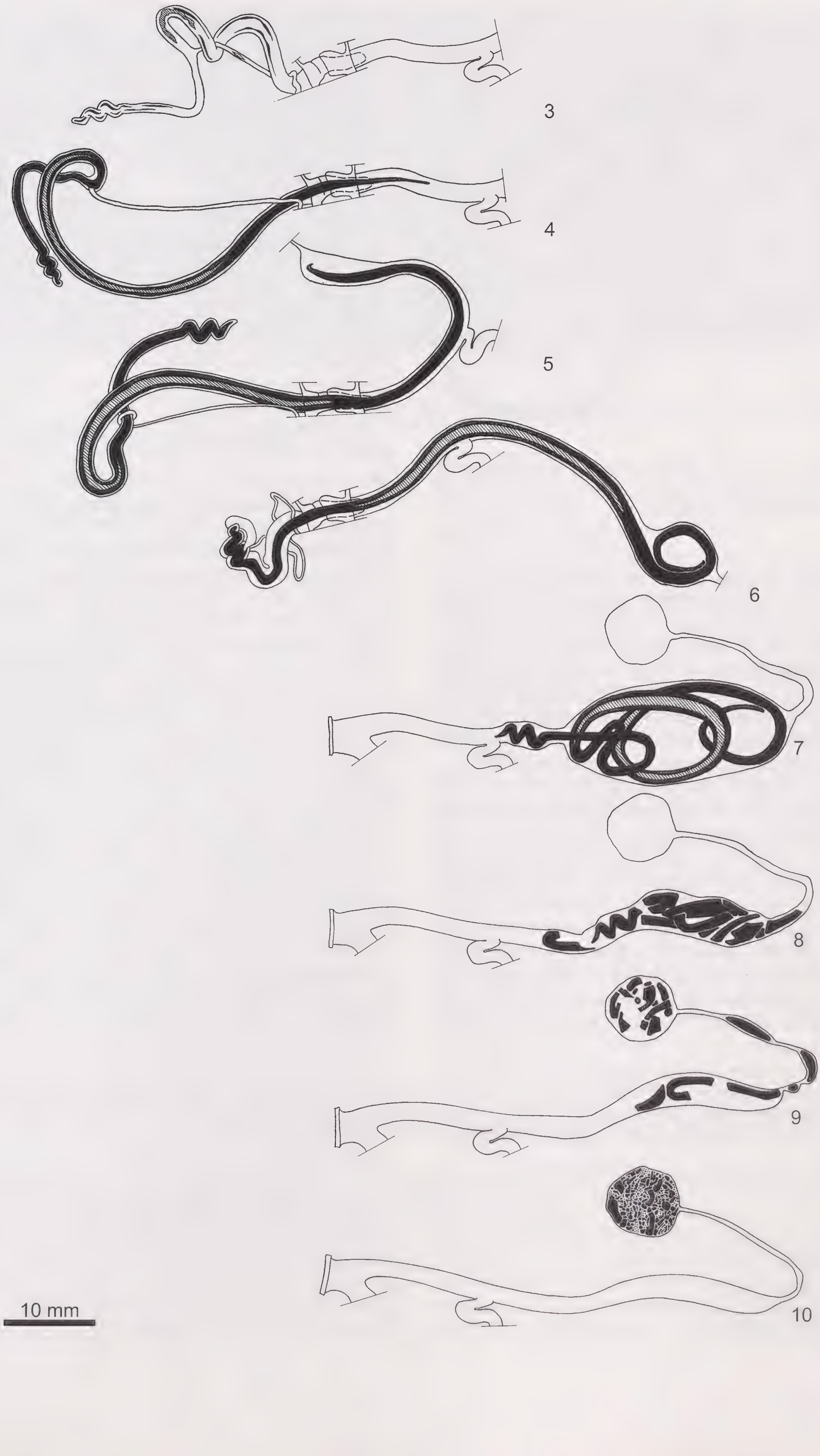
FIGS. 3–10. Consecutive stages (see text for descriptions of each stage) in copulation and sperm transfer in *Amphidromus inversus*. FIGS. 3–6: Stages 1–4 take place during mating; FIGS. 7–10: Stages 5–8 after coitus has ended (these latter four stages only show the recipient's genitalia). Although fertilization is simultaneously reciprocal, for simplicity, only one donor-recipient genital complex is shown, and parts of the genitalia that are irrelevant at a certain stage are truncated. The spermatophore material is shown in black, the sperm mass is indicated with hatching. All drawings are semi-diagrammatic and (with the exception of FIGS. 3, 6 and 8) composites of multiple dissections (FIG. 4 six dissections, FIG. 5 two dissections, FIG. 7 two dissections, FIG. 9 six dissections, FIG. 10 28 dissections).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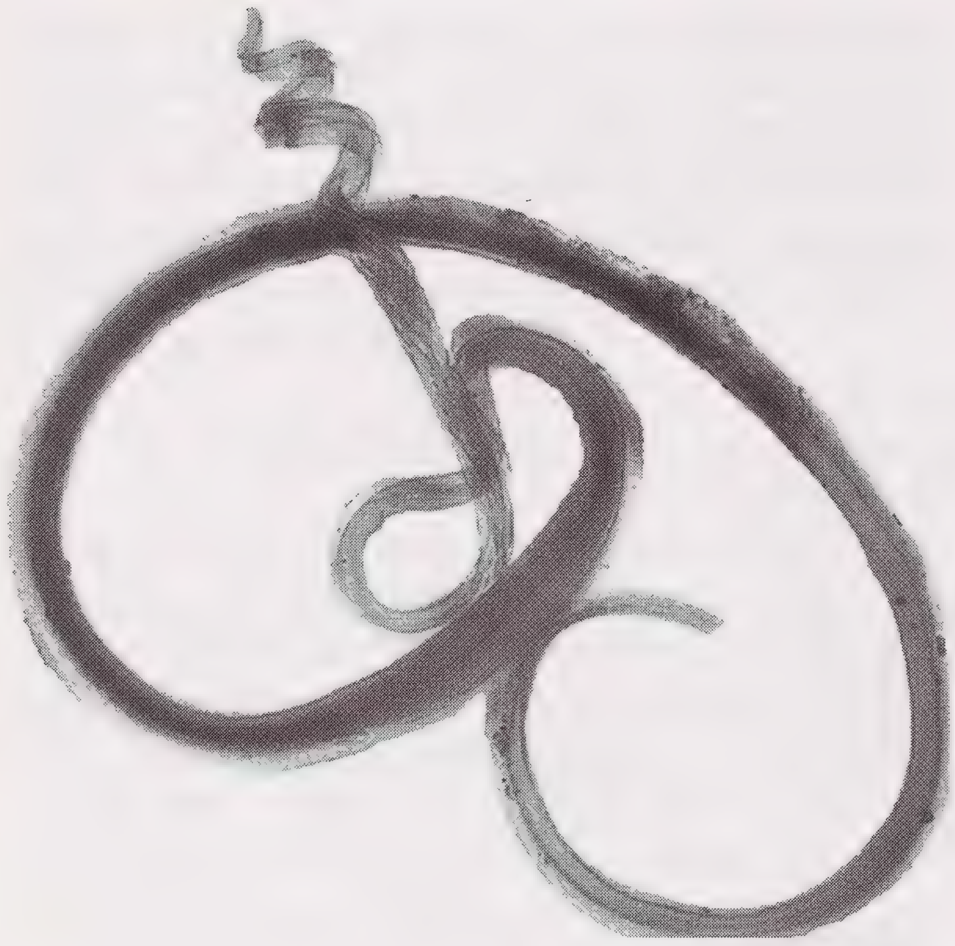


FIG. 11. A spermatophore from a sinistral individual of *Amphidromus inversus*; total length approximately 60 mm.

sperm, but has a narrow lumen that opens in a small hole just before the tip. The spermatophore shows several coils of varying tightness. The CES displays three corkscrew-like coils, followed by three much more tightly twisted coils in the neck of the CES, and then three very wide coils in the spermatophore body. The entire structure of the spermatophore is mirror-imaged in individuals of opposite chirality.

We obtained data on clutch size and coiling direction for 38 clutches of eggs and newly-hatched (but yet undispersed) juveniles (Fig. 5a, b; Table 1). Eggs were always found deep in rotting wood, under bark, and in cavities in tree trunks and branches. Five of the clutches were obtained from each of three sinistral and two dextral ovipositing snails, and in four of these, the offspring (which hatched around 40 days after egg laying) were of the same coiling direction as their mothers. Upon hatching, juveniles had approximately 1.5 whorls. Clutch sizes ranged from 2 to 70 individuals and were always invariant in coiling direction (with one exception; see caption to Table 1). The 12 same-clutch individuals that were sequenced, showed identical COI-sequences, one of which was deposited in GenBank under accession no. FJ472655.

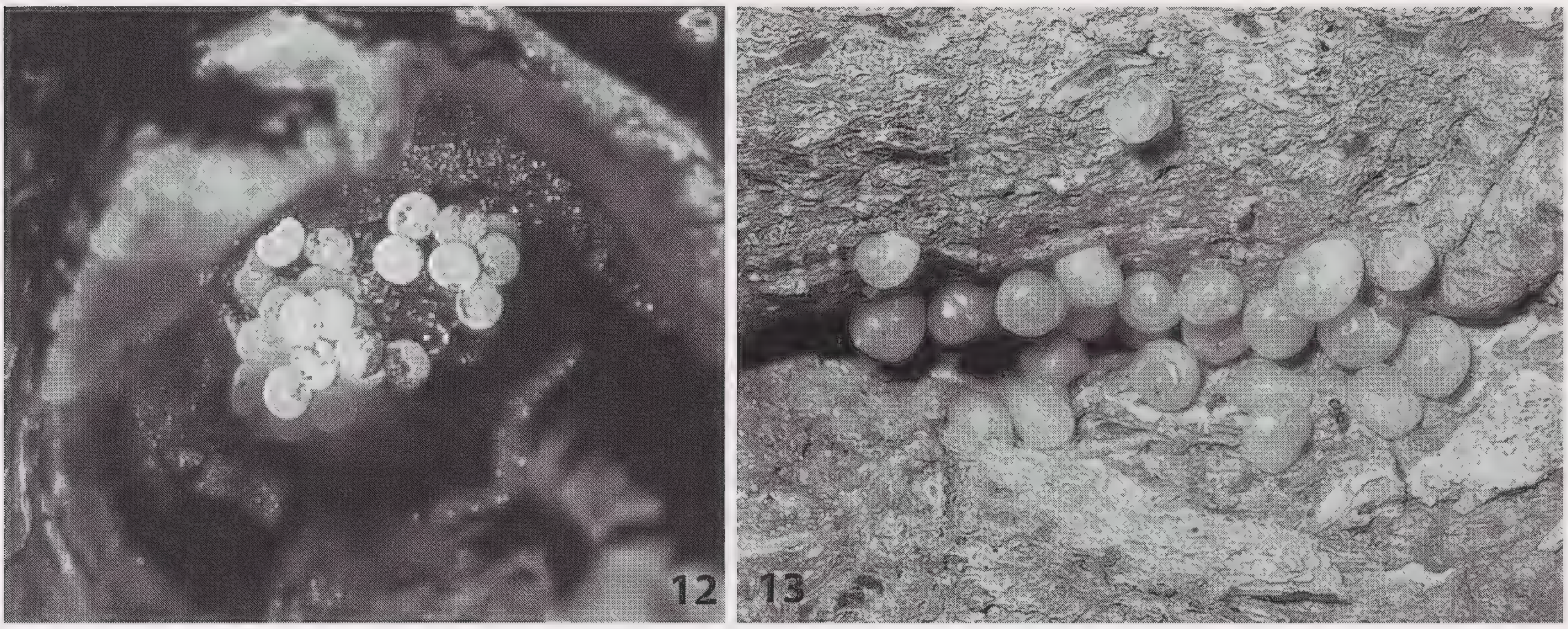
DISCUSSION

The mating behavior and functional anatomy of the genitalia in *Amphidromus inversus* correspond broadly to those known from other stylommatophoran gastropods (Tompa, 1984). However, some idiosyncrasies are also worth mentioning.

Since there is a tendency for tall-shelled stylommatophoran snails to copulate non-reciprocally and by shell-mounting (Asami et al., 1998; Davison et al., 2005; Davison & Mordan, 2007; but see Jordaens et al., 2009, who showed that the relationship does not hold if basommatophorans are also included), it was surprising to find that *Amphidromus inversus* (and, indeed, other species of the genus as well; Sutcharit et al., 2006) mate face-to-face and reciprocally. In fact, in all copulas studied, the positions of the genitalia and the spermatophore were exactly mirrored in both partners (a similar, exact reciprocity was also observed in *Mastus* by Parmakelis & Mylonas, 2002). In this respect, its mating behavior is similar to that of flat-shelled groups, and indeed, to most other, flat-shelled members of the Camaenidae (Davison et al., 2005; Davison & Mordan, 2007). It may thus constitute a plesiomorphic condition that was retained despite the change in shell shape. Even more surprisingly, the species appears to be unaffected by the generally great impediments to inter-chiral mating. If anything, this species gives preference to inter-chiral over intra-chiral mating (Schilthuizen et al., 2007).

TABLE 1. Data on egg clutches found in the field, in captive animals in Edinburgh Zoo, and ex situ in a garden in Kota Kinabalu. One mixed clutch, presumably the result of two ovipositions in the same locality (Schilthuizen et al., 2007), was treated as two separate clutches.

	Dextral offspring (total)	Number of clutches	Sinistral offspring (total)	Number of clutches	Average clutch size	Clutch size standard deviation
Kapas	170	10	475	24	19.0	12.7
Edinburgh Zoo	-	-	86	3	28.7	13.0
Garden enclosure	-	-	20	1	-	-



FIGS. 12, 13. Eggs and hatchlings of *Amphidromus inversus*, photographed in the field. FIG. 12: Clutch of recently deposited eggs; FIG. 13: Clutch of recently hatched juveniles, all sinistral; actual diameter of eggs and juveniles, approximately 3 mm.

Neither the mating position nor the interaction of the genitalia during copulation provide obvious clues as to why these obstacles do not play a role in this (and presumably other *Amphidromus* s. str.) species. Sutcharit et al. (2006) reported that in *A. atricallosus*, penes are extended several mm during mating, and that this may facilitate inter-chiral mating. However, in the copulations in *A. inversus* that we observed, the body walls of the partners appeared to be as close together as in the copulation of other snail species. Inter-chiral copulas tended to have their shells aligned, with the left side of the head of the sinistral partner against the right side of the head of the dextral partner. Intra-chiral copulas tended to copulate in a face-to-face manner, with the same side of the head touching in both partners. We suspect that there are, in fact, no intrinsic mechanical reasons why inter-chiral copulation is normally hindered in pulmonates. Rather, the chiral nature of courtship (Lipton & Murray, 1979; Davison et al., 2008) may normally prevent it, since it is adapted to bringing together the genital openings of individuals of the same chirality, and chiral courtship behavior probably has been modified or has become less stereotyped in *Amphidromus* to allow it. Detailed laboratory studies of courtship in *Amphidromus* as compared to chirally monomorphic relatives may clarify this.

We observed several apparently normal copulations in which at least one of the partners was conchologically immature, lacking a fully-formed apertural lip. Clearly, these individuals were reproductively mature. Mating and attempted mating involving conchologically juvenile individuals has been reported from other pulmonates as well (Tompa, 1984; Webb, 1951).

The sequence of events in spermatophore production, deposition, and digestion, appear to be similar to other stylommatophoran pulmonates. The one copula that we arrested at the very start of mating, shows that the spermatophore is built *de novo* in the epiphallus (the thickest part) and the flagellum (the thinner tail), after the donor's penial verge has been everted and inserted into the recipient's vagina. Since we do not know at what time this couple initiated mating, the time until the beginning of spermatophore formation cannot be determined, but based on what we know from other species, it is likely to be short: in *Helix pomatia*, its formation begins less than a minute after initiation of copulation (Lind, 1973), and in *Arianta arbustorum*, after five minutes (Baminger & Haase, 2001). In *Amphidromus inversus*, sperm starts entering the epiphallus via the vas deferens at the same time as the spermatophore begins forming. That is, the spermatophore is not, as in, for example, Milacidae (Wiktor, 1987), built empty and filled afterwards. Spermatophore formation takes up most of the duration of the copulation, although deposition in the recipient begins before spermatophore formation is complete.

The spermatophore's CES remained in the coiled tip of the flagellum in eight fixed copulas collected around mid-day, although the spermatophore head was already beginning to be pushed into the basal part of the recipient's gametolytic duct by a shortening of the donor's epiphallus. Only in one copula, fixed at the very end of the copulation (early evening), did the spermatophore begin to move out of the donor's male genitalia to be deposited entirely into the recipient's gametolytic duct. These observations correspond with studies in, for example, *Arianta arbustorum*, where it was also found that spermatophore formation took up over 80% of the mating time (Baminger & Haase, 2001).

Deposition into the gametolytic duct involves the folding of the long, coiled spermatophore into the thick-walled basal part. Initially, the corkscrew-shaped CES remains at the entrance of the gametolytic duct. As reported earlier (Schilthuizen et al., 2007), the tip of the CES (which carries the hole through which sperm is released from the spermatophore) is directed into the free oviduct after an inter-chiral copula, which could enhance sperm uptake in an inter-chiral mating, as compared with an intra-chiral one. The coils may, however, have further implications. Coils in the spermatophore occur in many unrelated land snail taxa. A few of the many examples are *Arianta*, *Mastus*, *Tandonia*, *Milax*, and *Thapsia* (Baminger & Haase, 2001; Parmakelis & Mylonas, 2002; Wiktor, 1987; Winter, 2008) and the springy quality with which they provide the spermatophore might help resist the peristalsis and the closing of the gametolytic organ, allowing more opportunity for spermatozoa to escape from it. Once the entire spermatophore is enclosed in the gametolytic duct, it is fragmented, presumably by a combination of enzymatic weakening and peristalsis in the rather muscular-looking basal part, and the fragments are then transported via the thin stalk to the gametolytic sac for digestion.

Finally, our data on egg clutches and mtDNA data suggest that each egg clutch had been deposited by a single mother snail (with the exception of one clutch, composed of 23 dextral and 2 sinistral juveniles, which may be the result of oviposition by two mother snails in the same place). As in other stylommatophoran pulmonates, the coiling direction of the offspring are identical, which fits the expectation that it is determined by their mother's genotype.

In conclusion, our observations show that intra-chiral as well as inter-chiral copulation and reproduction in the antisymmetric *Amphidromus inversus* do not conspicuously deviate from systems in other stylommatophoran pulmonates, as might have been expected from their characteristic chiral dimorphism. We expect that the obstacles for inter-chiral copulation in directionally asymmetric snails are behavioural, rather than mechanic (see also Jordaens et al., 2009), and that in *Amphidromus inversus*, these are overcome by small adjustments in the orientation of the partners. The evolution of the coiled spermatophore morphology may prove to be an interesting object of further study. The coils in the spermatophore tip may initially have evolved as a "pressure absorber" to resist the contractions of the gametolytic organ and, as a side-effect, have provided an advantage to inter-chiral copulations because of the better alignment between the donor's spermatophore tip and the recipient's oviduct. As a counter-adaptation, the length of the gametolytic organ may have increased to make the spermatophore lodge further away from the opening (Koene & Schulenburg, 2005), with a resultant further addition of coils in the spermatophore tail. Since *Amphidromus* s. str. species vary greatly in the number and shapes of coils in the CES (Sutcharit & Panha, 2006b), future work may be aimed at understanding spermatophore and gametolytic organ evolution across the subgenus in a phylogenetic framework.

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DOES SHELL ACCUMULATION MATTER IN MICRO-SCALE LAND SNAIL SURVEYS?

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INTRODUCTION

Land snails are most often surveyed to generate inventories with sites often less than 1 ha in extent and sampling taking place in microhabitats within the site that researchers consider likely to harbor snails (Cameron & Pokryszko, 2005; Menez, 2001, 2007). The most cost effective approach to site-level sampling has been suggested as a combination of bulk sampling of litter/soil and time restricted search (Emberton et al., 1996). This requires a single visit to each site, and the combination of methods enables the investigator to maximize the likelihood of finding both micro (< 5 mm) and macro (≥ 5 mm) snails (Sólymos et al., 2007).

This approach often relies on dead shells as a probabilistic indicator that a given species is present (Thurman et al., 2008) because several constraints (remote locations, investigator availability, weather conditions) may not enable surveys to be undertaken during the species' activity periods. Assessing species presence on the basis of dead shells can be realistic, given the rather sedentary and cryptic behavior of land snails, and in most cases gives comparable results at the site scale (Rundell & Cowie, 2003).

Recently, there is an increase in surveys that are addressing microhabitat-scale ecological differences regarding the relative abundances and species compositions of communities (e.g., under logs vs. leaf litter) within a site (Kappes et al., 2006; Sólymos & Páll-Gergely, 2007). Taphonomic issues (e.g., differential preservation of shells in different microhabitats) and biases introduced by sampling methods with different selectivity to dead specimens might become increasingly important as spatial scale decreases.

We carried out a field experiment in dolines (sinkholes, large karstic depressions) of the Aggtelek karst area, Hungary, to investigate the interacting effects of aspect (heat load) and microhabitat on land snail assemblages. Here we focus on the methodological issues raised by a stratified sampling design applied at the microhabitat scale. We aimed to determine the most straightforward strategy for comparisons among microhabitat types, that is, leaf litter, live trees, dead trees (here, equivalent to coarse woody debris) and rocks. We investigated how rates of shell disintegration and sampling method influence our interpretations of microhabitat-land snail community relationships.

MATERIALS AND METHODS

We surveyed 16 dolines at the Alsó-hegy plateau of the Aggtelek National Park, northern Hungary, August 16–18, 2007. The plateau and the dolines were forested and mainly covered by hornbeam (*Carpinus betulus*) and beech (*Fagus sylvatica*). The base rock was Triassic (Wetterstein) limestone. Soils of the dolines were rendzinas on the rims and plateaus in-between them, and brown forest soils on the slopes and in the bottoms of the dolines; soil pH (H_2O) in these soils is close to neutral, slightly basic (6–7.5) (Tanács & Barta, 2006). The dolines were 0.5–2 ha in extent. We collected land snails from four microhabitat types: litter, live wood, dead wood (i.e., coarse woody debris), and rock. In each doline, seven litter microhabitat samples were taken along a north-south transect, and three samples were taken in each of the other microhabitat types. We collected 1 L of litter per sample ("litter sampling" for short) and employed a time-restricted search (five minutes per replicate) in a 1 m radius around the litter sample location. Litter samples were collected adjacent to live wood, dead wood and rocks, and not from the wood or rocks themselves. Litter samples in the litter microhabitat were not collected near wood or rocks but that

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TABLE 1. Total number of individuals (fresh + broken, percentages for rows are given in parentheses) partitioned according to sampling method, microhabitat type and adult body size categories.

Method and microhabitat	Adult body size	
	Large	Small (< 5 mm)
Timed search		
Dead wood	51 + 14 (67.1 + 18.4)	11 + 0 (14.5 + 0.0)
Litter	33 + 28 (50.8 + 43.1)	3 + 1 (4.6 + 1.5)
Live wood	18 + 16 (48.6 + 43.2)	2 + 1 (5.4 + 2.7)
Rock	49 + 136 (25.9 + 72.0)	2 + 2 (1.1 + 1.1)
Soil-plus-litter sampling		
Dead wood	26 + 29 (11.7 + 13.1)	92 + 75 (41.4 + 33.8)
Litter	52 + 62 (8.7 + 10.3)	219 + 267 (36.5 + 44.5)
Live wood	63 + 56 (22.7 + 20.2)	74 + 84 (26.7 + 30.3)
Rock	108 + 98 (19.6 + 17.8)	211 + 134 (38.3 + 24.3)

the live wood, dead wood, and rock samples. Altogether, 32 samples (16 litter samples + 16 timed samples) were taken per doline.

Snails were identified to species and categorized according to extent of shell deterioration. Distinction of live animals and fresh shells was not feasible due to the delay in sample processing of litter samples, so these were combined and constituted the "fresh" group. Whitened, disintegrating and broken shells constituted the "broken" group.

Here we use the data from two dolines for which the samples have been fully processed. The 64 samples were grouped according to sampling method and microhabitat, and the species were grouped according to adult size – major shell dimension: smaller or greater than 5 mm; Kerney et al., 1983; semislugs were grouped together with small species – and family-group – Clausiliidae, Helicoidea, Zonitidae s. lat. (including Oxychilidae, Pristilomatidae, Daudebardiidae), and others.

We used the proportion of broken shells per sample in a logistic regression (binomial GLM) to determine its relation to sample type (method, microhabitat) and species specific category (adult size, taxonomy). The state of snails in a sample being either "fresh" or "broken" is a random binomial process with number of broken shells over total number of individuals as the probability of being broken and total number of individuals as the number of independent trials. We used the R software (R Development Core Team, 2008) for the computations. Data processing was done using the mefa 2.0 (Sólymos, 2008) R package. The full data set and the data analytic code are provided by Sólymos & Kemencei (2008).

RESULTS

The total number of fresh and broken shells differed both among microhabitat types and sampling methods. Most broken shells were found in rock and fewest in dead wood microhabitats. The numbers of small species were consistently lower in timed searches than in litter samples (Table 1).

The proportion of broken shells per sample was significantly associated with sampling method, microhabitat type and their interaction (null deviance: 88.9, df = 61; residual deviance: 55.8, df = 54; AIC: 287.4; Table 2). The proportion of broken shells was highest in the rock and lowest in the dead wood microhabitats. The litter sampling resulted in higher proportions of broken shells than the timed searches. However, the interaction showed that the proportion of broken shells in the dead wood microhabitat was lower in timed searches than in the litter sampling, whereas it was higher in rock microhabitats. There was no significant interaction with sampling method in the litter and live wood microhabitats (Table 2).

The proportion of broken shells per species was significantly associated with taxonomy and body size (null deviance: 26.8, df = 30; residual

TABLE 2. Logistic regression coefficients for the proportion of broken shells per sample. Effect sizes of the reference categories of factors (dead wood for microhabitat, and timed search for method) are included in the intercept.

Covariates	Estimate	Std. Error	p-value
Intercept	-1.6917	0.2908	< 0.001
Microhabitat			
Litter	0.8846	0.3667	< 0.05
Live wood	0.9140	0.4128	< 0.05
Rock	1.3772	0.3116	< 0.001
Method			
Litter sampling	0.9334	0.3142	< 0.01
Litter microhab. × Litter sampling	-0.7272	0.3915	0.063
Live wood × Litter sampling	-0.8381	0.4419	0.058
Rock × Litter sampling	-1.4839	0.3426	< 0.001

deviance: 14.2, df = 25; AIC: 134.9; Table 3). The proportion was high in the large bodied families, and highest among helicoids. Small sized zonitids (s. lat., *Daudebardia*, *Vitrea*) exhibited lower proportions of broken shells than larger sized zonitids (s. lat., *Oxychilus*, *Aegopinella*).

DISCUSSION

Our study reconfirmed that there is no universally appropriate and unbiased method for sampling land snails. We showed again that timed search and soil-plus-litter sampling is biased towards opposite ranges of the body size continuum (Cameron & Pokryszko, 2005; Sólymos et al., 2007). Besides selectivity for different size classes, shell accumulation rate also differed according to sampling method. We found that proportions of dead shells were higher for litter sampling than for timed searches, and higher proportions of small species are correlated with higher proportions of dead shells as opposed to live snails.

Proportions of dead shells also varied according to taxonomic group. Helicoids tended to accumulate more (i.e., disintegrate less) than other groups. This can be attributed to their relatively large, thick shells compared to zonitids or other smaller sized and thinner shelled taxa (Menez, 2002). Clausiliids, however, showed lower proportions of broken shells relative to helicoids, although their shells are also

TABLE 3. Logistic regression coefficients for the proportion of broken shells per species. Effect sizes of the reference categories of factors (other for family and large for size) are included in the intercept.

Covariates	Estimate	Std. Error	p-value
Intercept	-0.9454	0.1509	< 0.001
Family			
Clausiliidae	0.2886	0.2233	0.196
Helicoidea	0.4715	0.1765	< 0.01
Zonitidae s. lat.	0.2292	0.1852	0.216
Size			
Small	0.2406	0.1602	0.133
Zonitidae s. lat. × Small	-0.5343	0.2529	< 0.05

durable and relatively large. Their association with coarse woody debris might result in more rapid decay due to high fungal and microbial activity in woody debris. Thus, the observed species and size specific differences in shell accumulation may be linked to the species' microhabitat preferences.

When surveying at a fine, that is, microhabitat scale, different shell accumulation rates make among-microhabitat comparisons problematic. We found that shell accumulation rate was lowest in dead wood and highest in rock microhabitats. This may be related to the longevity and relative extent of these structural elements, with coarse woody debris decaying completely in up to 110 years (Holeksa et al., 2008; Lombardi et al., 2008; Rock et al., 2008), whereas rocks are an almost permanent and large feature in these forest habitats. Shell accumulation was intermediate in the live wood and litter microhabitats. Live trees might stand for a similar length of time as coarse woody debris, but dead wood provides good shelter and a food source during the relatively short time frame of intermediate decay stages when snails are able to retreat beneath the bark. Thus, woody debris can be considered as a more dynamic component than live trees (Jönsson et al., 2008).

Although we performed our study in a karstic area, where soil pH is neutral, slightly basic (6–7.5; Tanács & Barta, 2006), the effects of soil pH on shell disintegration in different microhabitats might add another level of complexity to our findings. Coarse woody debris has slightly positive effect on pH (Kappes et al., 2007), while on acidic soils, leaf litter can considerably buffer soil pH (Wäreborn, 1970). The increase of soil pH can also be expected adjacent to limestone outcrops. At the same time, high proportions of empty shells might also influence soil pH. These may result in differential shell disintegration, too.

Although dead shells might be good indicators of the occurrence and abundance of single species (Thurman et al., 2008), or when comparing species richness across sites (Rundell & Cowie, 2003), they pose considerable problems in microhabitat-scale comparisons. We suggest that the effect of shell accumulation should be assessed prior to any analysis at micro-scales. If the proportion of dead to live shells differs significantly among microhabitats, the most appropriate method is to use fresh (living) specimens only, and thus make the results more comparable. Further, because of the complex interaction between sampling method, microhabitat and taxonomic group, we suggest the use of timed search over litter sampling. Timed search is more cost effective, and can result in higher number of replicates. Living specimens can also be identified more easily, and released in the field, although vouchering is essential in order to permit future verification especially for difficult to identify species. In addition to microhabitat scale timed searches, it is advisable to collect soil-plus litter samples at the whole habitat scale to gain information on small sized species. Based on these complementary approaches, specific size classes (i.e., < 5 mm) could be excluded from timed search results to enhance comparability at the microhabitat level. This approach would result in both a maximized species list at the habitat scale and detailed abundance data for micro-scale comparisons. The methodology adopted should be geared to the research question being addressed.

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ON THE EGG MASSES, EGGS AND EMBRYOS OF *NOTOCOCHLIS ISABELLEANA* (D'ORBIGNY, 1840) (GASTROPODA: NATICIDAE) FROM NORTHERN PATAGONIA

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INTRODUCTION

Naticids are common predators in sandy bottoms of shallow waters around the world. Probably due to their infaunal habitat, they are hard to find alive, particularly in the Patagonian waters of Argentina. Nevertheless, their presence is confirmed by the typical predation boreholes that can be seen on their bivalve prey along the coast (e.g., Bromley, 1981; Carriker, 1981; Pastorino & Ivanov, 1996; Signorelli et al., 2006). These boreholes record information on predators without actually seeing them. Paleontologists have exploited this field, analyzing the behavior and producing models of predator-prey relationships in ancient and recent communities (Kabat, 1990, and citations herein).

Studies on the spawn of naticids are scarce in modern literature, despite its common presence in sandy bottoms. The typical sand collars were recognized several decades ago as belonging to this family. Ankel (1930) and Thorson (1935, 1940, 1946), among others, described these characteristic egg masses from different areas around the world. An accurate account of the morphology of the collar and capsules, including the way the gastropod builds them, was described by Giglioli (1955) and Ziegelmeier (1961). An interesting approach was recently published by Huelsken et al. (2008), who reviewed the naticids from Giglio, an Italian island off the coast of Tuscany. They compared mitochondrial and nuclear gene fragments (COI, 16S, H3, 18S) from adults and embryos from the egg masses, leaving no doubt about the mother species of each egg mass.

During the past few years, several papers were published in which egg capsules of various gastropods from the southwestern Atlantic were described (Pastorino & Penchaszadeh, 1999, 2002; Pastorino et al., 2007; reviewed in Gallardo & Penchaszadeh, 2001). However, there is no published information about naticids from the southwestern Atlantic. Similarly, the systematics on the group is far from complete. Pastorino (2005) mentioned 13 species of Naticidae living in the Patagonian coast and subantarctic islands. One of them, *Notocochlis isabelleana* (d'Orbigny, 1840), usually known under the genus *Natica*, is frequently found in the sandy infralittoral of northern Patagonia and southern Brazil. It actually ranges from Rio de Janeiro, Brazil, to Golfo Nuevo, Valdés Peninsula in the Argentine province of Chubut and is a typical representative of the Argentine malacological province.

In this paper, the egg mass, egg capsules, eggs, and embryos of *Notocochlis isabelleana* are described. A comparative table with data on other naticid spawn from different geographic regions is provided.

MATERIAL AND METHODS

Thirty complete egg masses of *N. isabelleana* were collected in the subtidal sandy bottom of the following localities: Caleta Falsa, San Antonio Oeste, Rio Negro, 40°47'S, 64°50'W (April 2008); Puerto Pirámides, 42°35'S, 64°16'W (November 1992, October 1993); Punta Pardelas, 42°37'S, 64°15'W (October 2007); Punta Este and Playa Paraná, Puerto Madryn, Chubut, 42°47'S, 64°57'W (October 2000 and April 2008) by SCUBA diving in ~5–10 m of depth. Most of them were complete, although a few were crushed during transport rendering the provenance of pieces impossible to establish. Collar measurements were done on complete egg masses only. The general shape of the collars was recorded, including the margins. Apical and basal diameters and thickness were measured with calipers.

There is a reasonable doubt about the exact identification of the egg masses, because none of them were collected when the animal was

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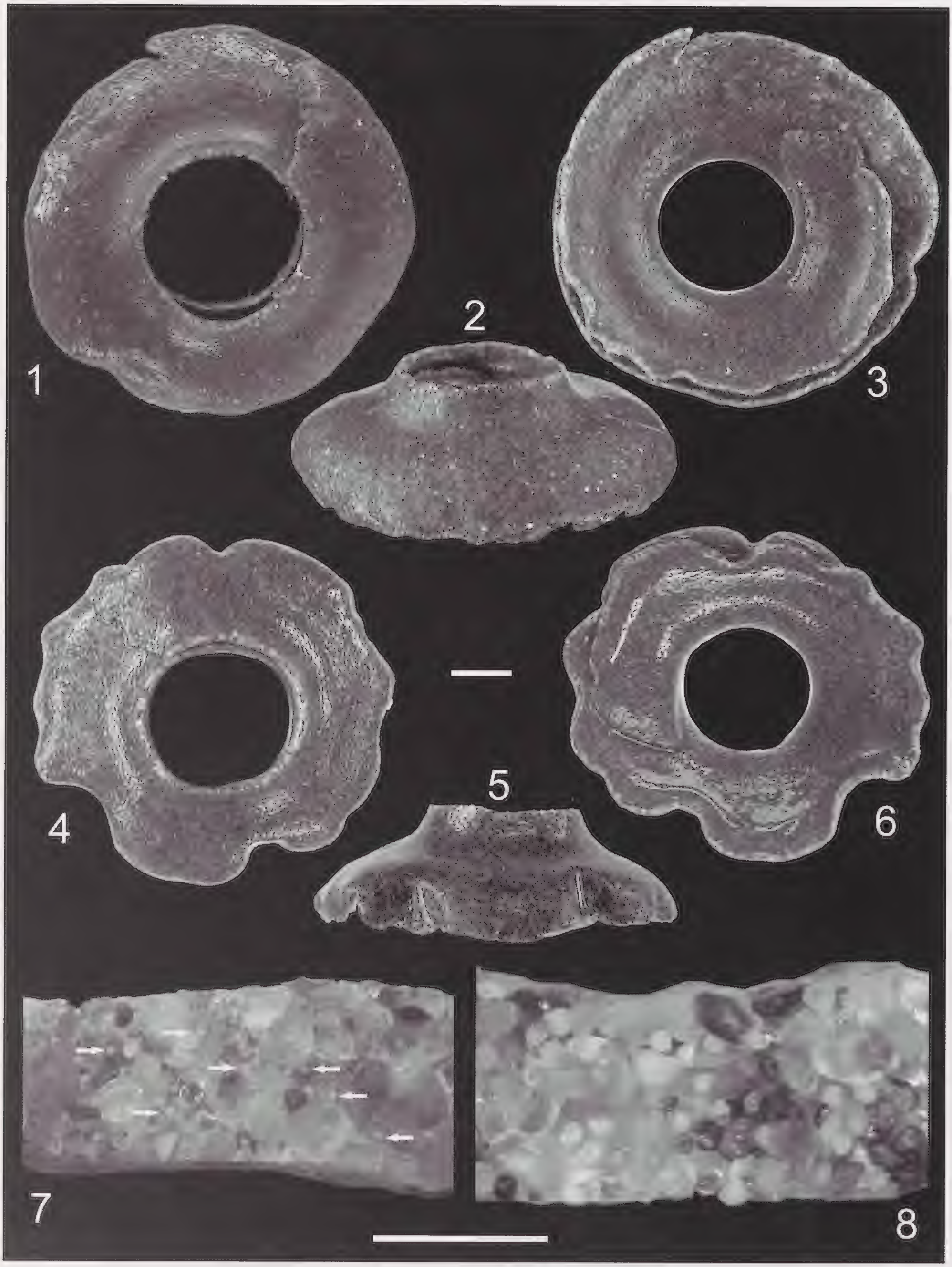
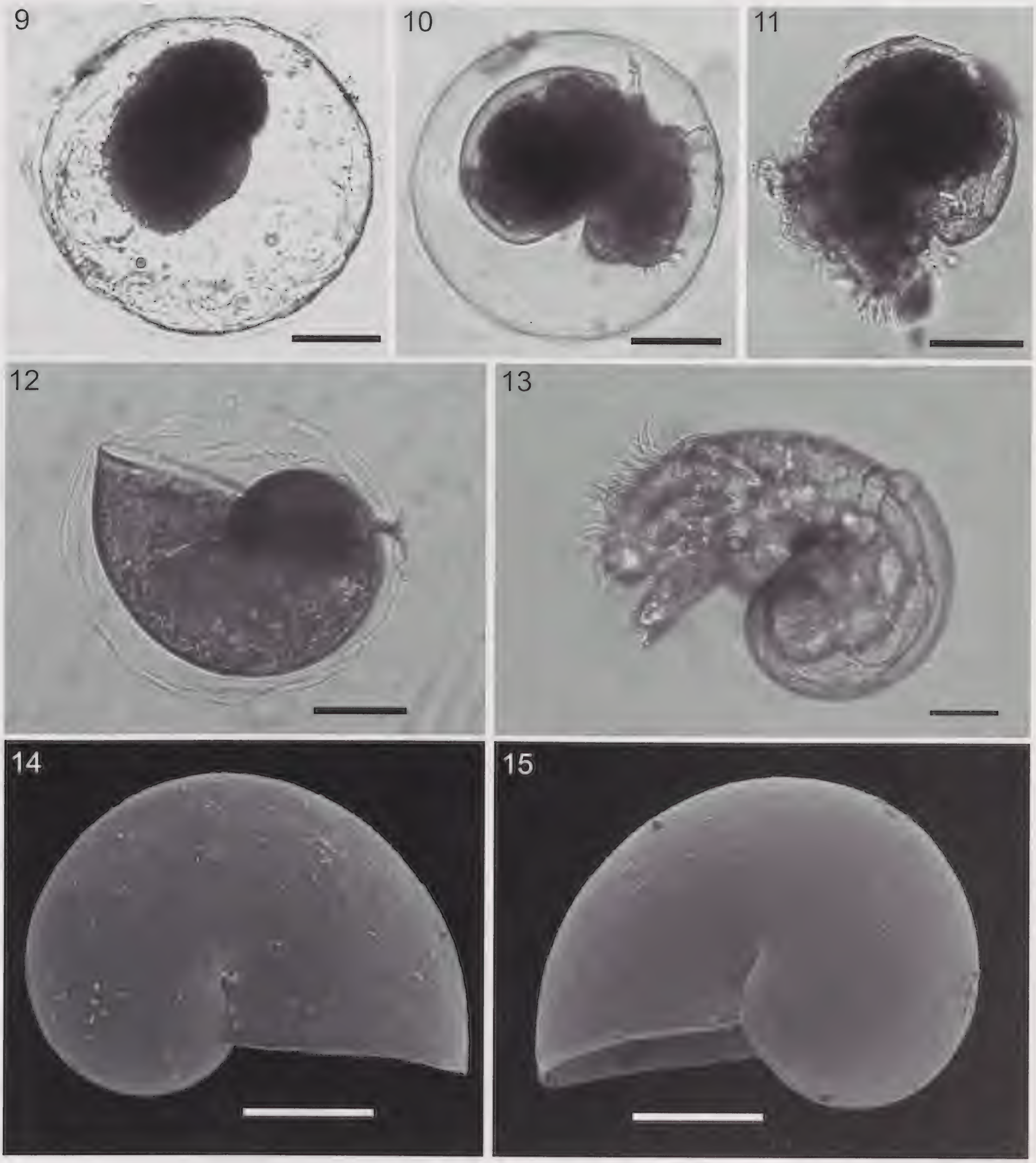


FIGS. 1–8. Egg masses of *Notocochlis isabelleana*. FIGS. 1–6: Apical, basal and lateral view of two egg masses of *Notocochlis isabelleana* (d'Orbigny, 1840); FIG. 7: Section of the collar showing the arrangement of embryos (arrows) among the sand grains; FIG. 8: Section of the collar with embryos with shell developed. Scale bars = 1 cm.

actually spawning. However, *N. isabelleana* is the only naticid living in the area and particularly at the depth where the material was collected. Similarity in the morphology and size of the egg masses, eggs, and embryos confirmed that all the spawns here studied belongs to this species.

Egg capsules, eggs, and embryos from all spawns representing different stages of development were measured under a microscope, with a 0.01 mm precision ocular micrometer and a Zeiss Axio Imager Z.1 microscope. Scanning electronic microscope images of the embryo shells were taken at the Museo Argentino de Ciencias Naturales (MACN) with a Philips LX 30. The main developmental features of each stage were described.



FIGS. 9–15. Embryonic stages. FIG. 9: Morulae; FIG. 10: Early "veliger" (intracapsular); FIG. 11: Late "veliger"; FIG. 12: Embryonic shell at hatching stage, FIG. 13: Veliger hatched larvae; FIG. 14: SEM of an embryonic shell, apical view; FIG. 15: SEM of another embryonic shell, basal view. Scale bars = 50 μm.

TABLE 1. Egg capsules, egg and embryo sizes of *Notocochlis isabelleana* (d'Orbigny, 1840) in µm.

	Egg capsule diameter	Uncleaved egg diameter	2-cell stage	8-cell stage	Morula	Early "veliger"	Shelled hatchling
Mean Size	185.2	97.6	148.3	132.4	123.4	133.3	151.5
SD	15.1	10.2	9.6	9.4	5.4	8.3	9.6
Range	153.7–235.3	73.5–114.4	119.1–162.9	109.2–153.4	112.4–136.4	118–150	137.7–167.7
n	202	24	38	61	55	32	24

RESULTS

Spawn consists of a 1–1.5 whorl spiral collar made of sand grains of two different diameters, the basal larger than the apical (Figs. 1–6). Compared to other members of the family, the collar is large. Basal diameter ranges between 35–67 mm, with an average of 45 mm (n = 23). Apical diameter is 14–28 mm, with and average of 19 mm. Thickness in its middle part never exceeds 2.5 mm (X = 1.9). The basal margin is always plicated and the apical one smooth.

The egg capsules are small and thin, of 185.21 ± 15.07 µm (X ± SD; N = 202) in maximum diameter, suboval in shape. The distribution on the collar around sand grains apparently never follows a specific pattern. Nevertheless, the egg capsules seem to be situated in the middle of the sand ribbon, between two layers of sand grains that keep them somewhat isolated from the outside (Figs. 7, 8). Each egg capsule hosts a unique round whitish egg. The diameter of the uncleaved egg measures 97.57 ± 10.2 µm (X ± SD; n = 24). We found the following stages according to the number of cells developed: two cells (148.34 ± 9.55 µm; n = 38), eight cells (132.4 ± 9.35 µm; n = 61), morulae (123.35 ± 5.43 µm; n = 55) (Fig. 9), and early "veliger" (133.28 ± 8.31 µm; n = 32) (Figs. 11, 12).

At hatching, a free swimming planktotrophic larva emerges, with one pair of eye spots, as well as statoliths, a bilobed unpigmented velum, and a corneous operculum (Fig. 13). The larval shell measured 151.48 ± 9.60 µm (X ± SD; n=24) maximum length (measurements in Table 1).

DISCUSSION

Giglioli (1955) proposed a classification of several naticid egg masses, with the aim of recognizing them in the absence of the spawning female. Classification by external morphology resulted in two large groups: thick (more than 1.5 mm thick) with rigid walls, and thin (less than 1.5 mm thick) with flexible walls. The first group has collars of 60 mm maximum height and the second of more than 85 mm. Each group includes two other divisions according to the morphology of the basal margin, smooth or plicated.

Gohar & Eisawy (1967) studied the egg masses of two naticids from the Red Sea, *Polinices mammilla* (Linnaeus, 1758) (as *Natica (Mamma) mamilla*) and *Mammilla melanostoma* (Gmelin, 1791) (as *Natica*). They criticized Giglioli's classification of the collars, because it is not completely applicable to the latter species. Essentially these authors agree with the external classification and disagree with the internal arrangement of the masses.

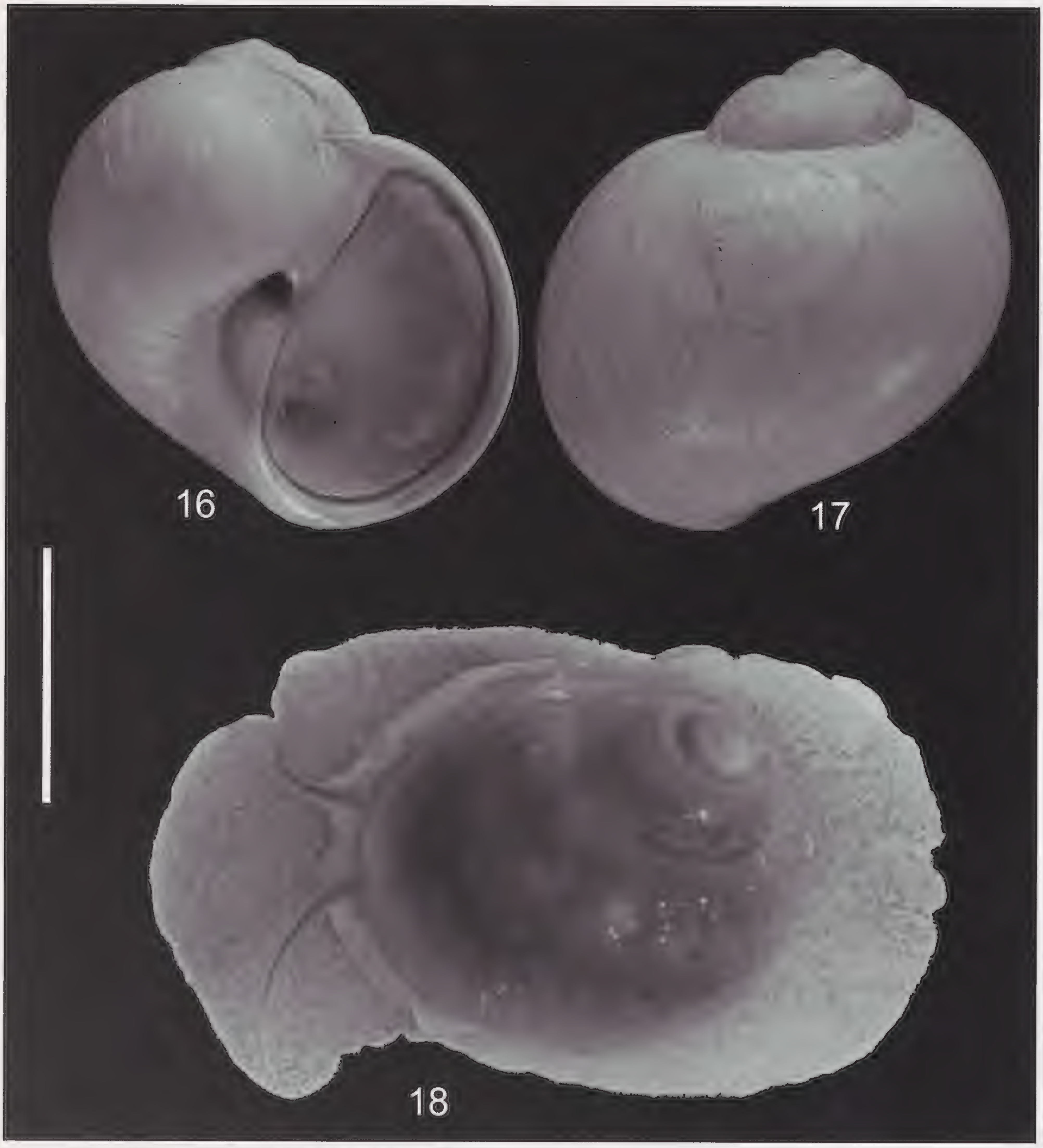
According to Giglioli's (1955) classification of the egg masses, *N. isabelleana* belongs in the "thin" group, with walls generally less than 2 mm of thickness.

There is only one egg of approximately 100 microns of maximum diameter in each capsule. No extraembrionic food and no nurse eggs were recorded. The egg capsules are very small, with little, completely translucent liquid. The embryo hatches as a planktotrophic veliger larvae with a maximum shell length of 185 microns. The size of the uncleaved egg diameter and therefore the small size of the free larvae could result in an extended period in the plankton. In fact, this contrasts with species of this family from other latitudes where either complete intracapsular development with large egg diameters (e.g., *Cryptonatica clausa*, a circumboreal species) have been described, or else with nurse eggs (e.g., *Euspira catena* from the North Sea and Mediterranean). As most of other studied naticid species (Thorson, 1940; Giglioli, 1955; Table 2), only one embryo per capsule develops in *N. isabelleana*.

According to Pedersen & Page (2000), naticids hatch in three ways: two with swimming larvae, planktotrophic and lecithotrophic, and

TABLE 2. Comparison of spawn, egg capsules and hatchling shells measurements of naticids. Sources are: Thorson, 1935(1), 1940(2); Giglioli, 1949(3), 1955(4); Natarajan, 1957(5); Fioroni, 1966(6); Gohar & Eisawy, 1967(7); Ziegelmeier, 1961(8); Bandel, 1975(9), 1976(10); Pedersen & Page, 2000(11); Kingsley-Smith et al., 2005(12); Huelsken et al., 2008(13).

Species and source	Collar size (in mm)		Egg capsules				Larval shell length (in µm)
	Basal diameter (apical)	Thickness	Diameter (in µm)	Embryos per capsule	Nurse cells	Emergent form	
Amauropsis islandica (Gmelin, 1791)(1)	?		1,500–1,750	1	No	Crawling juvenile?	750
Cryptonatica clausa (Broderip & G. B. Sowerby I, 1829)(1)	40		2,250	1	No	Crawling juvenile	1,500
Euspira catena (da Costa, 1778)(3), (6)	73 (47)	1.5–2.2	1,425–1,925	2–4	Yes	Crawling juvenile	800
Euspira heros (Say, 1822)(3)	25–73	0.9–1.1	630–850	4–84	No	Veliger	130–150
Euspira lewisii (Gould, 1847)(4), (11)	195 (75)	2.0–2.3	190–260	1	No	Veliger	180
Euspira nitida (Donovan, 1803)(8), (12)	30		208–0.254	1	No	Veliger	190–200
Euspira pallida (Broderip & G. B. Sowerby I, 1829), as *E. groenlandica* (Möller, 1842)(1)	45 (12–13)		2,500–3,000	1	No	Crawling juvenile	1,500–1,750
Euspira triseriata (Say, 1826)(3), (4)		1	850–1150	1–3	No	Crawling juvenile	500
Glossaulax didyma (Röding, 1798), as *G. ampla* (Philippi, 1848)(2)		1		1		Crawling juvenile	600–700
Mammilla melanostoma (Gmelin, 1791)(7)	70	0.6–1.6				Veliger	200
Natica livida Pfeiffer, 1840(9), (10)	15–17	0.7		3–6		Veliger	
Natica marochiensis (Gmelin, 1791)(5)	35–50	0.45–0.52	183–300	3–7	No	Veliger	100–116
Natica tigrina (Röding, 1798)(5)	56–79	0.8–1.1	251–284	1		Veliger	200–250
Natica trailli Reeve, 1855(2)		1		1		Veliger	310
Natica vitellus (Linnaeus, 1758), as *N. rufa* (Born, 1778)(2)		0.5–1		1	No	Crawling juvenile	800
Naticarius canrena (Linnaeus, 1758)(9), (10)	80–120	1.0	400	1		Veliger	560
Neverita duplicata (Say, 1822)(4)		1.0–1.2	190–250	1	No	Veliger?	180
Neverita josephinia Risso, 1826(13)	51–59			1		Crawling juvenile	780
Notocochlis isabelleana (d'Orbigny, 1840)	45 (19)	< 2.5	185	1	No	Veliger	151
Polinices hepaticus (Röding, 1798)(9), (10)	40–45		200–300	1		Veliger	280
Polinices lacteus (Guilding, 1834)(9), (10)	23–43 (37–40)		100	1		Veliger	400
Polinices mammilla (Linnaeus, 1758)(7)	36–80	1		1		veliger	130
Tectonatica sagraiana (d'Orbigny, 1842)(13)	24–40			1		Veliger	300



FIGS. 16–18. Adult of *Notocochlis isabelleana* (d'Orbigny, 1840). FIGS. 16, 17: Two views of a shell; FIG. 18: Living adult with the foot extended. Scale bar = 1 cm.

crawling juveniles. *Notocochlis isabelleana* hatches as planktotrophic larvae. This could explain, at least in part, the wide geographic distribution of the species along the southwestern Atlantic coast, although Johannesson's (1988) study proved at least in part that transport of benthic stages other than planktonic free larvae could be a dispersal factor.

Gallardo & Penchaszadeh (2001) postulated that sand environments in the southwestern Atlantic have a large percentage of species with an encapsulated complete development, and hatchlings are crawling juveniles. *Notocochlis isabelleana* (Figs. 16–18) constitutes an exception to this pattern, despite its clear association to soft bottoms.

The morphology of the egg masses of *N. isabelleana*, as in all Naticidae, shows a peculiar adaptation to soft bottoms. The shape of the collar fixes the whole spawn to the bottom, allowing, however, good aeration. In addition, the wavy basal margin is apparently a very effective deterrent for the accumulation of sand that could hamper water circulation and ventilation of embryos and eggs during development (Gohar & Eisawy, 1967).

As far as we can see in sections of the sand collar, there is a selection of the place where most of the egg capsules are settled. Thus, the capsules in the middle of the sand grains gain some protection.

Table 2 shows different spawn measurements of naticid species from several regions. The problem comparing these species is the unresolved taxonomy. Studies on the phylogeny of the family are needed, and the relationship among genera is far from understood. *Notocochlis isabelleana* has an extremely small veliger shell, only comparable to several species of *Natica* s. l. (i.e., *N. marochiensis*), *Neverita*, *Polinices,* and *Euspira* (i.e., *Neverita duplicata*, *Polinices mammilla* and *Euspira lewisii*), despite the fact that these genera belong in different subfamilies. In addition, the same table also shows that all species with a crawling juvenile have a larval shell length larger than 310 microns, and those with free veliger larvae are smaller. As can be seen in the literature, nurse eggs are not the rule among the naticids or at least they are not as common as in the Muricidae. Most of the known species have only one embryo per capsule, however some showed a very variable number (i.e., *E. catena*, *E. heros* and *N. marochiensis*).

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